ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH

1960

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

ANNUAL REPORT of Program activities 1960

TABLE OF CONTENTS

Title	Principal Investigator
The Director's Report	Masland
Direct Training	Wipf
Extramural Programs	Hove
I. Research Grants	Hove
II. Training Grants	Hartman
III. Special Fellowships	Hartman
Perinatal Research Branch	Berendes
Biometrics Branch	Goldstein
Epidemiology Branch	Kurland
Laboratory of Perinatal Physiology	Windle

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346
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1960

Intramural Research - 1960

Scientific Director's Report---------- Shy

Clinical Research

Clinical Director's Report---------- Baldwin

Branch of Electroencephalography and Clinical Neurophysiology

Summary---------- Ajmone-Marsan

Individual Project Report--

Serial No.	Title	Principal Investigator
1(c)	Studies on the problem of epilepsy with emphasis on focal seizures----------	Ajmone-Marsan
2(c)	Pathologic findings in patients with "centrencephalic" electroencephalographic patterns----------	Ajmone-Marsan
3(c)	Pathological and electroencephalographic analysis of metastatic tumors of the brain----------	Strang Ajmone-Marsan
4(c)	Depth electroencephalography----------	Van Buren Ajmone-Marsan
5(c)	Cortical control of sensory imput----------	Ajmone-Marsan Morillo
6(c)	Analysis of callosal responses in the visual cortex of cat----------	Morillo

Medical Neurology Branch

7(c)	The Histopathological and Chemical Investigations of Neuromuscular Disorders---	Shy
8(c)	The Physiology and Pharmacology in Myasthenia Gravis----------	Shy
9(c)	The Use of Monoamine Oxidase Inhibitor as an Anti-convulsant Medication for Centrencephalic Seizures----------	Smith Prockop

Intramural Research - 1960

Scientific Director's Report ..... Ray

Clinical Research

Clinical Director's Report ..... Bertrand

Branch of Electroencephalography and Clinical
Neurophysiology

Summary ..... A.Jasper-Marsan

Individual Project Reports--

Serial No.	Title	Principal Investigator
1(c)	Studies on the problem of epilepsy with emphasis on focal seizures	A.Jasper-Marsan
2(c)	Pathologic findings in patients with "centrencephalic" electroencephalographic patterns	A.Jasper-Marsan
3(c)	Pathological and electroencephalographic analysis of metastatic tumors of the brain	Strang A.Jasper-Marsan
4(c)	Depth electroencephalography	Van Buren A.Jasper-Marsan
5(c)	Cortical control of sensory input	A.Jasper-Marsan Morrillo
6(c)	Analysis of cortical responses in the visual cortex of cat	Morrillo

Medical Bioscience Branch

1(c)	The Histophysiological and Chemical Investigations of Neuromuscular Disorders---	Ray
2(c)	The Physiology and Pharmacology in Myasthenia Gravis	Ray
3(c)	The Use of Monomine Oxidase Inhibitor as an Anti-convulsant Medication for Centrencephalic Seizures	Dalton Procop

Serial No.	Title	Principal Investigator
10(c)	Cytological Studies of Neuromuscular Phenomena in Patients with Neuromuscular Disease and in Experimental Animals	Engel
11(c)	Native State of Bacterial and Viral Desoxyribonucleic Acid (DNA)	Cummings
12(c)	Electrolytes and Energy Metabolism in Normal and Epileptogenic Cerebral Cortex in Vitro	Tower
13(c)	Metabolism of Free and Protein-Bound Amino Acids in Normal and Epileptogenic Cerebral Tissues	Tower
14(c)	Isolation, Fractionation, Characterization and Metabolism of Brain Nucleic Acids (RNA and DNA)	Sporn Dingman
15(c)	Muscle Proteins in Neuromuscular Diseases	Horvath
16(c)	Study of muscle cholinesterase and its inhibitors	Irwin
17(c)	Interaction between macromolecules and pharmacologically active chemicals	Irwin
18(c)	Neurohumoral factors in Cystic Fibrosis of the Pancreas	Eyerman Irwin
19(c)	The localization of muscle fibers in a single motor unit	Norris
Ophthalmology Branch		
Summary		von Sallmann
Individual Project Report		
21(c)	Effects of Chronic Administration of Cortico-steroids on the Lens	von Sallmann
22(c)	Studies of the Incorporation of H^3 Thymidine in the Corneal Endothelium of the Rabbit	von Sallmann Grimes

Serial No.	Title	Principal Investigator
23(c)	The Study on Mitosis in the Corneal Endothelium----------	von Sallmann
24(c)	Studies of the Effect of High Energy Proton Microbeams, Simulating Cosmic Primaries, on the Mouse Lens----------	von Sallmann Curtis
25(c)	Study of Ocular Toxoplasmosis and its Therapy----------	von Sallmann van Alphen
26(c)	Basic Factors in Refraction Anomalies-------	van Alphen
27(c)	The Ocular Toxicity of 1-phenyl-2-Hydrazinopropane (JB-516)----------	Jones
28(c)	Clinical Investigations on Rare Diseases of the Eye 1. Epiphora and Nasopharyngeal Tumor 2. Corneal Opacities in a Patient with Cryoglobulinemia and Reticulohistiocytosis----------	Cohan Oglesby
29(c)	Corticosteroids as a Cause of Cataract in the Human----------	Oglesby von Sallmann
30(c)	Clinical Glaucoma Study----------	Okun von Sallmann
31(c)	Studies on the Activity of Cells in the Eye of the Limulus----------	Fuortes
32(c)	Organizational Aspects of Vertebrate Retina Determined Electrophysiologically----	Gouras
33(c)	Electrophysiological and Photochemical Relationships in the Vertebrate Retina------	Gouras
34(c)	The Adaptation of Electrical Enhancement Techniques to Clinical Electroretinography--	Gouras
35(c)	Design and Construction of Ophthalmic Instruments----------	Gunkel

Serial No.	Title	Principal Investigator
36(c)	Functional Studies in Retinal Anomalies and Diseases (Electroretinography, Adaptometry, and Perimetric Light Sense Studies)----------	Jones Gunkel
37(c)	Increment Thresholds in a Subject Deficient in Cone Vision----------	Rushton Fuortes
38(c)	Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision-------	Rushton
39(c)	Dark Adaptation and Regeneration of Rhodopsin----------	Rushton
40(c)	Investigations on Spectral Sensitivity of Diurnal Squirrels. (ERG Reactions of Pure Cone Mammalian Retinae)----------	Tansley Copenhaver
41(c)	Some Aspects of Electroretinographic Responses in the American Red Squirrel------	Tansley Copenhaver Gunkel
42(c)	Some Observations on the Off-Effect of Mammalian Cone Electroretinogram----------	Tansley Copenhaver Gunkel
43(c)	A Study of the Proteins of the Lens---------	Resnik
44(c)	The Structure of Ganglioside----------	Resnik
45(c)	The Rhodopsin Cycle in the Developing Mammalian Eye----------	Bonting Caravaggio
46(c)	Membrane ATP-ase and Aqueous Formation------	Bonting Simon
47(c)	Influence of Alpha Chymotrypsin on the Healing of Corneal Endothelial Wounds-------	Huckel
48(c)	Electron Microscope Study on the Protozoon Toxoplasma gondii----------	Wanko

Serial No.	Title	Principal Investigator
49(c)	Electron Microscope Studies on Biopsies of Human Muscle Disease----------	Wanko Shy
50(c)	Electron Microscopic Studies on Tissues of the Eye, such as Epithelium, Fibers and Capsule of the Lens, Epithelium of the Ciliary Body, the Optic Nerve and the Conjunctiva----------	Wanko
51(c)	The role of the vasculature in the maintenance of intraocular pressure---------	Macri
52(c)	Study on the Pharmacodynamics of Various Agents Affecting Intraocular Pressure-------	Macri
53(c)	Observation of the Flow Pattern in the Ciliary Processes of Albino Rabbits. An in vitro study----------	Scullica
Surgical Neurology Branch		
Summary----------		Baldwin
Individual Project Report		
54(c)	Epileptogenic Mechanisms in the Brain of Man and Other Primates----------	Baldwin
55(c)	Functional Representation in the Temporal Lobe of Man and Higher Primates----------	Baldwin
56(c)	Studies of Involuntary Movements----------	Van Buren
57(c)	Effect of Lesions upon the Function and Structure of the Human Central Nervous System----------	Van Buren
58(c)	A Study of the Functional Anatomy and Pathology of the Human Visual System--------	Van Buren
59(c)	Effect of Radio-Frequency Energy on Primate Brain Mechanisms.----------	Baldwin
60(c)	Study of Inhibitory Interneurons in the Cerebral Cortex----------	Li

Serial No.	Title	Principal Investigator
61(c)	Cortical Intracellular Potentials in Response to Stimulation of the Geniculate Body----------	Li
62(c)	Basic Mechanisms of Electrical Activity Recorded from Surface of Brain----------	Li
63(c)	Basic Mechanisms of Single Cell Discharges in Cerebral Cortex----------	Li
64(c)	Physiological Studies in Tissue Culture Material----------	Li Klatzo
65(c)	The Effects of Deep Hypothermia in Higher Primates----------	Baldwin
66(c)	Study of Deep Hypothermia in Dogs----------	Galindo
67(c)	Psychological Evaluation of Temporal Lobe Operations----------	Lansdell
68(c)	Observations on Vascular Permeability in the Brains of Rats Exposed to Alpha Particle Radiation----------	Klatzo
69(c)	Study on Pinocytosis in the Nervous Tissue--	Klatzo
70(c)	Study on Morphological Changes due to Exposure to Radio-frequency Energy----------	Klatzo
71(c)	Study of Vascular Permeability in the Central Nervous System using Fluorescein Labeled Serum Proteins (FLSP) Technique-----	Klatzo
72(c)	Histochemical Study on Central Core Disease----------	Seitelberger
73(c)	Observations on Alterations in the Vascular Permeability in the Experimental Allergic Encephalitis----------	Seitelberger
74(c)	Investigation of the Etiology, Pathology and Clinical Manifestations of Cerebral Palsy and Allied Conditions; Also of the Epilepsy During Childhood----------	Dekaban

Serial No.	Title	Principal Investigator
75(c)	Maternal Condition During Pregnancy and the Course of Birth in Relation to Neurological Abnormalities in the Infants and Pathologic Lesions in Products of Abortion----------	Dekaban
76(c)	Pathological Lesions in the Central Nervous System Occurring During Prenatal, Intranatal and Early Postnatal Life----------	Dekaban
77(c)	The Incidence and the Type of the Central Nervous System Abnormalities Encountered in Offspring Born to Diabetic Mothers----------	Dekaban Baird
78(c)	Measurements of External and Internal Orbital Distance in Males and Females from Birth to Adulthood----------	Dekaban
79(c)	Preparation of the Horizon of the Normal Development of the CNS in Mice and Experimental Production of Congenital Malformations of the CNS---	Dekaban
80(c)	Study of Chromosomes in Patients with Congenital Malformation and in Mongoloids----------	Dekaban Bender

Laboratory of Neuroanatomical Sciences

Summary----------		Palay

Individual Project Report

NINDB-NS-NC-1	Ultrastructure of nervous system------	Palay
NINDB-NS-NC-2	The role of γ-aminobutyric acid in the metabolism of the central nervous system----------	Albers
NINDB-NS-EN-3	The pathogenesis of myelopathies------	Cammermeyer
NINDB-NS-EN-4	The functional significance of oligodendrocytes----------	Cammermeyer

Serial No.	Title	Principal Investigator
NINDB-NS-EN-5	"Light" microscope studies of normal and diseased neurons----------	Cammermeyer
NINDB-NS-DR-6	The specificity of the sympathetic nervous system in the regulation of pupillary function----------	Guth
NINDB-NS-DR-7	Heterogeneous reinnervation of the diaphragm----------	Guth
NINDB-NS-DR-8	Regeneration of peripheral nerves-----	Feringa
NINDB-NS-DR-9	Regeneration in the peripheral nervous system: Selectivity of pathways and specificity of terminations----------	Guth
NINDB-NS-DR-10	Aspects of Experimental Neuro-embryology----------	Guth
NINDB-NS-DR-11	Regeneration in the spinal cord of cats and monkeys----------	Feringa
NINDB-NS-DR-12	Studies to determine the possible role of auto-immune antigens in cerebral scar formation----------	Feringa
NINDB-NS-DR-13	Studies of spinal cord regeneration in the newt----------	Feringa
NINDB-NS-DR-14	The neurological basis of color vision in the fish----------	Bernstein
NINDB-NS-DR-15	Gustatory function following hetero-geneous reinnervation of taste buds in the fish----------	Bernstein
NINDB-NS-FN-16	Further studies on the efferent component of the vestibular nerve-----	Gacek
NINDB-NS-FN-17	Comparative anatomy of the afferent and efferent auditory nerve connections in submammalian vertebrates----------	Boord
NINDB-NS-FN-18	The ascending and descending connections between the medial geniculate body and the auditory cortex----------	Rasmussen

Serial No.	Title	Principal Investigator
NINDB-NS-FN-19	A study of the auditory and vestibular afferent and efferent systems including the receptors--------	Rasmussen
NINDB-NS-FN-20	Study of the afferent extrinsic and intrinsic neuronal connections of the medial geniculate body----------	Morest

Laboratory of Biophysics

Summary----------		Cole

Individual Project Report

NINDB-B-1	Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses----------	Adelman Binstock Kishimoto
NINDB-B-2	Ionic Permeabilities of Nerve Membranes: Theoretical Investigations-	FitzHugh Chandler
NINDB-B-3	Studies of Acetylcholinesterase Inhibition and Nerve Activity----------	Whitcomb
NINDB-B-6	Ionic Permeabilities of Nerve Membranes: Investigation of new methods and of other axons than squid----------	Moore Julian Goldman
NINDB-B-7	Ionic Permeabilities of Squid Giant Axon Membrane. Tracer and Electrical Experiments and Analyses----------	Adelman Mullins Sjodin Hodgkin

Laboratory of Neurochemistry

Summary----------		Trams

Individual Project Report

NINDB-NC-1	Biosynthesis of Sphingolipids----------	Brady Trams

Serial No.	Title	Principal Investigator
NINDB-NC-6	Biosynthesis of Aromatic Compounds-----	Brady
NINDB-NC-7	Metabolism of Inositol----------	Agranoff
NINDB-NC-14	Enzymatic Synthesis of Fatty Acids-----	Brady
NINDB-NC-15	Biosynthesis of Cholesterol----------	Agranoff
NINDB-NC-16	Role of Redox Reactions in Excitation--	Brady Spyropoulos
NINDB-NC-17	The Structure and the Biosynthesis of Brain Gangliosides----------	Trams
NINDB-NC-18	Glycolipids as Receptor Sites----------	Trams
NINDB-NC-19	Effect of Hydrocarbons on Enzyme Synthesis----------	Trams
NINDB-NC-20	Carboxylation of Acyl-CoA Derivatives--	Brady
Laboratory of Neurophysiology		
Summary----------		Marshall
Individual Project Report		
NINDB-NP-SS-5	The mechanism of nerve excitation------	Tasaki Spyropoulos Oikawa Arvanitaki Chalazonitis
NINDB-NP-SC-3	Generation of impulses in nerve cells--	Frank Nelson
NINDB-NP-SC-8	Generation of impulses in the Mauthner cell of the goldfish----------	Oikawa
NINDB-NP-SC-9	Studies of chronic changes in single nerve cells associated with learning---	Frank Nelson
NINDB-NP-SC-10	Intracellular recordings from inferior colliculus and medial geniculate in response to auditory stimulation----------	Nelson Erulkar

ANNUAL REPORT

Calendar Year 1960

National Institute of Neurological Diseases and Blindness
National Institutes of Health

The Director's Report

The past year has been one of unusual development within the Institute. Of long-range significance was the decision to establish an independent basic research program in neurological diseases within the intramural program. This, of course, necessitated a careful re-programing of all intramural activities. The allocation of funds to construct a new building for NINDB's research program makes it possible now to plan a well-rounded and carefully integrated total program of neurological research within the Institute. To execute the intramural program, Dr. G. Milton Shy has been appointed Associate Director for Intramural Research and Dr. Maitland Baldwin, Clinical Director.

The report of the Associate Director for Intramural Research shows that planning for the intramural program has been carried forward with thoroughness and imagination. In this newly developing program, the major emphasis will be in the general area of neurochemistry, molecular biology and neuropathology. The objective is to exploit to the fullest the newly discovered techniques in these important basic sciences, and to use them not only to advance basic knowledge of chemistry and the structure of the nervous system, but also to focus them upon the specific problems presented by the major neurological disorders.

The appointment of Dr. William F. Windle as Assistant Director of the Institute, with primary assignment to the perinatal laboratories in Puerto Rico, provided the opportunity to develop long-range planning of the role of this important facility. Located at the U.S. Public Health Service Station in San Juan, the colony of breeding animals now numbers nearly 200. This colony is proving a valuable resource for the basic research studies conducted by the staff and visiting scientists. The free ranging colony on Cayo Santiago continues to show a healthy development. It provides valuable background data on normal growth and development, and on the physical and social characteristics of animals living in this "natural" habitat.

The Institute has continued its investigations of unusual neurological disorders appearing in geographical isolates around the world. On the island of Guam the continuing investigations of amyotrophic lateral sclerosis have uncovered unusual neuropathological findings in this disease, and a relationship to Parkinsonism and presenile dementia, which suggest a similarity to Jakob-Creutzfeldt's disease. The Guam situation also affords a favorable opportunity to study certain forms of convulsive disorder which appear to have an unusually high incidence in that population.

Similarly, far-flung investigations of the bizarre neurological disease (kuru), uncovered in the Fore tribe of New Guinea, are being pursued. For these investigations, the intramural operation has served as a focus. Material and data from these studies are being given widespread distribution to highly specialized laboratories throughout the world.

INTERNATIONAL RESEARCH PROGRAM

The principal objective of the first or exploratory phase of NINDB's international program, with headquarters in Antwerp, Belgium, concentrated on the organization of the World Federation of Neurology (WFN) as an effective instrument and channel of professional communication for the implementation of collaborative neurological and sensory research programs throughout the world.

The balance of effort was devoted to a study of the most suitable geographic areas for an NINDB worldwide collaborative program of research development. In considering the countries in which programs could be most effectively launched and the types of programs to be promulgated, no weight was given to political, geopolitical, or diplomatic questions. Conclusions were reached on the basis of the geographic areas which would return the greatest research payload in the shortest time considering current facilities, promise for growth, supply of trained men, and professional enthusiasm.

More specifically, the aims of the program were: 1) Completion of the blueprint of the basic organizational structure of the World Federation of Neurology, a global federation of national professional neurological societies; 2) Organization of a permanent WFN Secretariat at the Institut Bunge in Antwerp; 3) Formation of WFN problem commissions composed of outstanding international scientists in special disciplines related to neurological and sensory disorders; 4) The inauguration of a monthly journal, WORLD NEUROLOGY, as the official publication of the Federation; 5) Establishment of an active liaison with other appropriate international health agencies and organizations; 6) Aid in the organization of international symposia, congresses, and assemblies devoted to health and research in these fields; 7) Study of the research potential for the development of collaborative international programs in various geographic areas; 8) Exploration of the feasibility of obtaining financial support for these programs from governmental or other agencies in countries other than the United States; 9) Activation of a cerebrovascular pilot study in geopathology, and the initiation of important field trips.

To date the realization of these aims and objectives may be summarized as follows:

1 - <u>WFN Society Membership</u>

The basic WFN blueprint has been finalized. The national neurological societies of 49 nations representing 10,000 neurologists and neurologic scientists are now members of the Federation. This number includes the societies of the U.S.S.R., all the Iron Curtain countries of Eastern

Europe including Yugoslavia; India, and countries in the Far East except North and South Korea. The only significant land masses not represented are some countries of the Middle East, Red China, Formosa, most of the new states of Africa, Bolivia, Ecuador, and Paraguay in South America, several countries in Central America, Mexico and the Irish Free State.

2 - Organization of Secretariat

The Secretariat of the WFN has now been organized on an efficient basis and operates from offices in renovated space on the top floor of the Institut Bunge, Antwerp, Belgium.

The Secretariat has the services of a full-time medical officer, Mark Callens, M.D.; an executive administrative officer, Colonel William R. Finks; and supporting secretarial staff. It also has a part-time professional staff (serving without pay) composed of Ludo van Bogaert, M.D., President, WFN; Armand Lowenthal, M.D., Director of the Department of Neurochemistry; and F. H. J. Radermecker, M.D., Director of EEG, Institut Bunge.

The Secretariat administers all the routine WFN affairs and serves as a coordination center for the exchange of information, scientific specimens, and the activities of its Problem Commissions. It also collects dues from member societies and manages the finances of the Federation.

3 - Activation of Problem Commissions

The 10 Problem Commissions of the WFN have been organized, are functioning, and each one has had at least one Commission meeting. These meetings are often followed by productive symposia on a specialized problem. To date, the organized Problem Commissions are in neurochemistry, neuropathology, comparative neuroanatomy, comparative neuropathology, biometry and genetics, developmental neurology, neuroradiology, neuroanesthesia, and the history of neurology. Three additional Problem Commissions (neurogenetics, ophthalmology, and tropical neurology) have been formed and will hold their inaugural meetings in 1961.

The Problem Commissions serve as integrative centers for specialized neurologic disciplines, exchange centers for scientific materials and literature, and formulators of research opportunities which are more susceptible to a single international effort than to the aggregate impact of several individual national efforts. Many of the planning functions of the Secretariat in Antwerp are being gradually decentralized to the Problem Commissions. The Commissions probably represent the hard core of the future of the WFN.

4 - Launching WORLD NEUROLOGY

The inauguration of the WFN official publication, WORLD NEUROLOGY, last July, has been successful in serving as an instrument for the exchange of information in four languages and for the implementation of concepts for world-wide collaboration. This journal presents original contributions and review articles by international authorities. Its circulation already has passed 3,000 and is rising rapidly.

5 - Liaison with Other Organizations

A workable liaison arrangement has been established with many other international health agencies such as the World Health Organization, the International Brain Research Organization (IBRO), the World Federation of Neurosurgical Societies, the World Federation of Mental Health, and the Council for International Organizations of Medical Sciences (CIOMS). Under the auspices of the Association for the Aid of Crippled Children, affiliation with interested voluntary groups in the United States and elsewhere is being pursued. Two meetings already have been held in New York with the executive and research directors of leading voluntary health agencies.

6 - Organization of Congresses and Symposia

The WFN Secretariat has made the administrative arrangements for the organization of the Seventh International Neurological Congress and the Fourth International Congress of Neuropathology, both to be held in September 1961, the former in Rome, the latter in Munich. It also has stimulated planning for a Pan-American Congress of Neurology and Neurologic Science. This will be the first of its kind, and will be held in Sao Paulo and Rio de Janeiro in 1963. The planning committee will be held in Sao Paulo in 1961.

The WFN has conducted many fruitful symposia on specialized problems raised by the Problem Commissions. A good example is the symposia held in Antwerp on paramyloidosis, November 1960. Clinicians and pathologists of Lisbon brought clinical histories and pathological material on this disease to Antwerp. The symposium was held in conjunction with a meeting of the members of the Problem Commission of Neurochemistry to determine a future plan of attack on the disorder.

7 - Promising Geographic Areas for Collaborative International Research

As a result of the survey of the director of NINDB's international programs, the countries now considered most promising on a world-wide basis for productive results in collaborative neurological and sensory disease research are: Latin America, especially Brazil and Argentina; Poland; and certain parts of India, particularly Bombay. These areas have considerable trained personnel in neurologic science, excellent physical facilities, and great enthusiasm. Most of them, however, lack a complete complement of trained manpower in one or two specialized

areas necessary for effective research. These shortcomings could be overcome were it possible to develop an exchange fellowship and professional program, both short- and long-term.

The types of research development most likely to be successful include epidemiological projects now underway which could be continued and implemented such as the WFN project on the geographic pathology of cerebrovascular disease, now in a pilot testing stage in Antwerp.

8 - Procurement of Financial Support from Other Nations

The question of financial support from countries other than the United States for international neurological research is difficult but does not appear to be impossible. A small number of contributions have already been procured and the possibility of obtaining some substantial support seems promising.

The Danish Multiple Sclerosis Society, following a meeting of the WFN Biometry and Genetics Commission in Copenhagen, now supports a project on the epidemiology of multiple sclerosis. The German Federal Republic in cooperation with WFN contributed to the costs of the field investigation of Professor G. Schaltenbrand (Wurzburg) on multiple sclerosis in South America. The Gulbenkian Fund contributed $1,000 to the paramyloidosis symposium in Lisbon. The Senate of the Republic of Argentine is introducing a bill to grant $15,000 for support of the meeting and symposium of the WFN Commission of Tropical Neurology (1961). This project will also receive support from the city of Buenos Aires, the University of Buenos Aires, the Argentine Neurological Society, the National Academy of Medicine and the National Institute of Research (Professor B. Houssay). The Muscular Dystrophy Associations of America have contributed $5,000 for the meeting and symposium of the WFN Problem Commission of Neurogenetics. L'IRSAC (Belgium) is contributing to the WFN project in the Congo (Uganda).

9 - Pilot Study - Geopathology of Cerebrovascular Disease

As of July 1, 1960, a pilot testing project in the geopathology of cerebrovascular disease, under the auspices of WFN (principal investigators: L. van Bogaert and J. Moossy--Grant No. B-2433), was activated. This is an evaluation study to find an objective and valid methodology for application to a later collaborative project in this field.

INFORMATION

The Information Office of NINDB produced a large number of materials during 1960 telling of needs and accomplishments in the field of neurological and sensory disorders.

These materials took the form of special reports, speeches, articles, press releases, exhibits, letters, pamphlets, motion picture films, and radio and television scripts. Some of these were designed to fill the needs of various governmental groups, including Congress. Other materials were directed toward members of scientific professions, and still others implemented a broad program of information for the general public.

In addition to the NINDB Director's special reports to Congress and the opening statement, the Information Office prepared articles, speeches and reports to fill requests from the White House, individual Congressmen, Congressional committees, the Department of Health, Education, and Welfare, the Public Health Service, the National Advisory Neurological Diseases and Blindness Council, and many voluntary health agencies.

During 1960, requests fulfilled included six speeches for Departmental and Congressional use; special reports for Senate and House Appropriations Committees on multiple sclerosis, cerebrovascular diseases, mental retardation, speech and hearing, vision, the Collaborative Project, and supplementary perinatal accomplishments, and an extensive report on international perinatal research for the U.S. Senate's Committee on Governmental Operations.

A report for NIH was prepared on "Channels of Communication for NIH Scientists" and contributions were made to various Departmental publications, including the DHEW Annual Report, DHEW Mental Retardation Report, Handbook of DHEW, PHS Programs for State Health Educators, Public Health Reports--Review of NINDB's Highlights, the DHEW Report on Known Statistics on Prevalence, Mortality, and Economic Loss From Neurological and Sensory Disorders, the NIH Scientific Directory and Annual Bibliography, and the National Science Foundation's Scientific Information Activities of Federal Agencies.

Pamphlets produced by the Information Office during 1960 for public distribution included "Mongolism--Hope through Research" (also brought out in a Spanish-language edition) and "Cerebral Palsy--Hope through Research." The Information Office aided the National Committee for Research in Neurological Disorders in producing "Exploring the Brain of Man," a 28-page booklet on general neurology published under an NANDB grant. The Office also produced for the Collaborative Project pamphlets entitled "You and Your Baby" and "Careful, Accurate Interviewing" and revised for the Public Health Service "Care of the Eyes" and "How to Protect Your Hearing."

In press and scheduled for release early in 1961 are "Parkinson's Disease" and "Cataract and Glaucoma." In final stages of production and scheduled for release in the spring of 1961 are "Highlights of Research for 1960," composed of 52 weekly reports of NINDB scientific advances and 21 program developments, and "The Fateful Months When Life Begins," a public pamphlet on the Collaborative Project.

Special material for both the scientific and lay press was produced during the year by the Information Office. This included the preparation and distribution of 14 press releases, 48 news and feature articles for the

NIH Record, which were made available to the press, and a press kit for the Collaborative Project. The Office also held a press conference for NIH Lecturer, Dr. Wilder Penfield, and is planning a press conference early in 1961 for members of NINDB's Mission to the USSR to survey the field of maternal and child care.

As an aid to science writers, the Information Office supplied background material on NINDB and arranged interviews for representatives of numerous periodicals and press services. Material also was prepared for three television programs.

During 1960, the Information Office replied to approximately 1,300 letters of public inquiries, including Congressional requests but not counting the large number of requests received for individual and bulk orders of pamphlets, and replied to more than 2,500 telephone inquiries from the public.

The Office edited and cleared many articles and scientific manuscripts (including the 152-page "Report of the Medical Exchange Mission to the USSR--Pharmacology and Physiology of the Nervous System), produced a new exhibit on the Collaborative Project entitled "The Fateful Months When Life Begins," revised an earlier exhibit on the Project entitled "50,000 Mothers and Their Babies," widely distributed two NINDB films ("Year of Birth" and "Neurological Examination of the Newborn"), and made arrangements for distribution of another film ("Neurological Examination of the One-Year-Old"). Nine NINDB exhibits had 29 showings during 1960 for a total of 177 show days before professional scientific societies, national and international medical societies, and allied health groups.

RESEARCH PROGRESS

There has been further clarification of the processes underlying the formation of blood clots in the vessels of the brain which are responsible for certain forms of stroke. We now know that the "Transient Ischaemic attack" is the result of arteriosclerotic narrowing of one or more large vessels supplying the brain and that these minor episodes, as well as the stroke which they presage, may be reduced by the use of anticoagulants. In certain cases, surgical removal of the affected vessels appears to offer a cure.

There has been further elucidation of certain diseases responsible for mental retardation. Additional new forms of inborn chemical disorders have been demonstrated, and for one (maple sugar urine disease) a promising dietary therapy has been developed. Chromosomal defects have also been found in a number of forms of mental retardation in addition to Mongolism.

The possible role of an "auto-immune" process in multiple sclerosis and other demyelinating diseases is still uncertain. However, liquefaction of the myelin sheath, a characteristic feature of the disease, has now been observed under the microscope in tissue cultures of nerve cells treated with specific immune serum. Modification of the "allergic" reaction in animals has been achieved by a special desensitization process.

The perennial question of a possible virus agent as a cause of multiple sclerosis has again been raised as a result of the discovery of infectious diseases in sheep and goats which resemble multiple sclerosis and are characterized by latent periods of as much as several years after infection.

Further clues regarding the chemical basis of some forms of Parkinsonism have been developed as a result of the discovery that certain tranquilizing drugs may produce transitory Parkinson-like symptoms. The enzyme systems which react with these drugs have now been shown to relate to manganese--an element long known to cause fatal Parkinsonism.

Migraine headaches have been shown to be associated with the liberation of vasomotor enzymes in the affected areas. A new drug with blocking effects offers promise of relief.

There have been several advances in our knowledge of neuromuscular disorders. The chemical basis of one baffling form of muscle disease--familial periodic paralysis--has now been established. In respect to myasthenia gravis, a new drug (galanthamine) may prove a useful addition to our means of control of paralysis.

Evaluation of diagnostic tests for glaucoma reveals that the tonographic test has distinct advantages for detection. Further advances relating to glaucoma have been made in understanding the disturbances of fluid production in the eye which influence the internal or intraocular pressure. In the evaluation of therapy for uveitis, the use of pyrimethamine has been proven effective in 43 percent of cases when the eye inflammation was caused by toxoplasmosis.

Continuing advances in the surgery of the ear are producing more effective relief for otosclerosis and for certain forms of vertigo. New diagnostic tests, which include actual recording of the electrical responses of the human ear, are now proving capable of clarifying the basic defects underlying hearing and language disorders. These tests point the way for greatly improved educational and training programs.

During the past year, this Institute has recognized certain particularly critical areas of need and has placed special program emphasis on them. These include cerebrovascular diseases, disorders of vision, neurological disorders of childhood, speech and hearing disorders, evaluation of therapy, and instrument development.

CEREBROVASCULAR DISEASES

Working closely with the Heart Institute in this general area, this Institute has focused attention on the need for more precise knowledge of the relationship of disturbances of cerebral circulation to neurological symptoms and for a critical evaluation of medical and surgical therapy.

Research progress and future needs relating to vascular disorders of the brain were outlined by international authorities in April of 1960 at a conference supported by this Institute and organized by the American Academy of Neurology. A summary of major conclusions, prepared by a small group of consultants immediately after the conference, clearly delineated special areas of research need and crucial, unanswered questions. This summary received wide circulation.

Another conference in January of 1961 reviewed subsequent data, which included new methods of evaluating the adequacy of circulation through the brain by the use of radioactive tracers and the refinement of angiographic techniques for determining the location of obstructions of the arteries to the brain. Meticulous studies were presented showing anatomical status of the blood vessels of brains of patients dying from cerebrovascular diseases. These studies provided further information regarding the frequent location of obstructive processes and clarifying the dynamic changes that relate to the sudden occlusions of these blood vessels resulting in stroke.

The final part of the conference was concerned with a review of Institute-supported studies regarding the role of anticoagulants in cerebrovascular disease. Research in this area is being carried forward with increasing momentum. The two cooperative Institute studies are providing a continuing stimulus and important background basic data on the natural history of cerebrovascular diseases and their response to treatment. Studies to date indicate that anticoagulant therapy, though hazardous, can delay the occurrence of strokes in selected cases. Continued studies of these groups are defining with increasing precision those cases suitable for therapy. Additional forms of treatment must also come under investigation in the near future.

An Institute committee of consultants is continuing to survey this field and is preparing an additional detailed status report for further guidance.

Stemming from heightened recognition of this important field, applications have been received by the Institute for the development of research centers focused on the problem of cerebrovascular disease. The opportunity to mount these broad, interdisciplinary attacks within the center program should initiate significant research.

DISORDERS OF VISION

The opportunity to expand research in problems of normal vision and diseases of vision is making possible specific control of some causes of blindness. At the same time research on glaucoma continues at our Bethesda laboratories, an Institute-supported cooperative study is seeking better methods of screening and diagnosing glaucoma. A Glaucoma Family Study by Institute grantees proves the heavier incidence of glaucoma among relatives of those already diagnosed. Also, a heavier incidence of glaucoma in persons with diabetes has been found. Another investigation showed that persons

known to have glaucoma provide an especially fruitful survey group for diabetes.

A May 1960 conference sponsored by Institute grants brought 34 participants from many fields to confer on diabetic retinopathy. Diabetics are living longer; diabetic retinopathy, which already accounts for 8.4 percent of all blindness, can be expected to increase until controlled by research discoveries.

A new approach to the baffling problem of uveitis, a major blinding disease, is a pilot study for analyzing records of uveitis patients. Initiated at an Institute-supported conference on uveitis, the pilot study may provide clues to causes, prevention, and cure. Successful treatment is reported in 43 percent of the large number of patients referred to the Institute with uveitis caused by toxoplasmosis infection.

NEUROLOGICAL DISORDERS OF CHILDHOOD

Another major program concern of this Institute is neurological disorders of infancy and childhood. These disorders include cerebral palsy, mental retardation, congenital malformations of the nervous system, and congenital blindness and deafness. Approximately 25 percent of the Institute's research appropriation is devoted to this area, the major focus of which is centered upon the Collaborative Project. The Project recently completed its second year of operation, after two and a half years of intensive preparation. About 5,500 expectant mothers and 4,500 babies were studied in the pretest phase, and as of December 31, 1960, approximately 13,500 expectant mothers and 8,000 babies had been enrolled in the main phase of the Project.

The Project will eventually involve some 50,000 expectant mothers and their children from all parts of the nation. Record keeping begins in early pregnancy and continues through labor and delivery into the early years of the lives of the children.

Working on the Project, at 15 private medical institutions across the country, are obstetricians, pediatricians, neurologists, and specialists in at least a dozen other medical and related scientific fields. All of them have a common goal: to gain new knowledge about the relationship between events and circumstances of pregnancy, labor, delivery, and the first month of life on the one hand, and the later occurrence of neurological or sensory disorders on the other. The common hope is that this new knowledge will make it possible to prevent these disorders and thereby enable every child to lead a full and useful life.

During 1960, developments of the Project included the revision of examination forms and manuals to further assure uniform collection of data on study mothers and babies; the development and adoption of methods for post-mortem examination of all abortions, stillbirths, and children dying in the first month of life; and the final preparation for the routine screening to begin in 1961 of blood samples from study mothers during

pregnancy for evidence of virus infections.

Only the most fragmentary clues are as yet available from the preliminary analysis of the early data now in hand. Information based on data submitted by the collaborating institutions indicates that the number of study children born with a neurological defect may be much higher than originally expected. A recent check of all data presently available on study children who have had a one-year neurological examination showed an incidence rate of one cerebral palsied child out of every 100 study children, or one percent. The original estimate, based on data from other studies, was one-half of one percent.

A serious problem relating to the conduct of research with severely neurologically damaged children results from the fact that many of them are under custodial care in isolated institutions for the retarded. This year, the Institute has organized a survey group to help plan and recommend means of strengthening research activities in such institutions.

At our Bethesda laboratories, research projects relating to neurological disorders of childhood include microscopic and chemical studies of the brain and central nervous system. Also, in the neuropathological laboratories, various studies concern gross and microscopic examination of the brains of children and adults known to have suffered from mental retardation. These observations in adults and children are being paralleled by animal experiments in which deliberate efforts to produce a "mentally retarded" animal have been undertaken.

Within the extramural program, grant-supported projects include studies on the chromosomes of Mongoloids, the toxic and metabolic deficiency disorders, certain developmental defects, and other studies relating specifically to mental retardation and cerebral palsy. Other investigations relate to asphyxia and lesions of the brain, tests of mentality, and mental development of premature infants who became blind with retrolental fibroplasia before its prevention was discovered.

DISORDERS OF SPEECH AND HEARING

Until recently, our ability to deal effectively with disorders of hearing and the closely associated defects of language was hampered by the lack of accurate diagnostic tests. Lack of comprehension due to deafness from ear disease has been extremely difficult to differentiate from inability to comprehend because of a defect of the central receptive mechanisms. This diagnostic difficulty has been a major obstacle to a clear understanding of the causes of language dysfunction and to the development of effective treatment and training programs.

Highly specialized tests in medical audiology now are solving the problem. The Institute's program has emphasized the training of scientists capable of developing and administering these new tests. The program has also included the application of these tests to the screening of large numbers

of normal and handicapped individuals.

Preliminary findings of a survey of hearing and hearing loss in 4,000 school children have been reported in a large-scale study which shows promise of future important results. The study is jointly supported by the Institute and the Children's Bureau. One result of this project has been the demonstration that audiometric testing alone cannot identify those physical abnormalities of the ear which may have predictive value in indicating hearing loss.

Similar studies of childhood hearing loss are being conducted by some States. For example, hearing tests of some 11,000 school children in Nebraska revealed that about six out of every 100 children had hearing problems. Although the difficulty of detecting hearing loss in infants has been a major obstacle, recent results indicate that the electroencephalograph may be a useful tool, since responses to sound can be determined by brain wave recordings.

A newly developed technique is making it possible for the first time to record from the human ear and auditory nerve electrical responses to sound. There is thus available a truly objective measure of "hearing" at this most fundamental level.

Through animal experimentation investigators now can trace the sound impulse from the ear throughout the brain. They can observe the modifications of this impulse at the various way stations within which it is "evaluated" by the brain.

Further evaluations of new techniques cited last year for relieving otosclerosis have continued this year. Numerous supported studies report a high degree of success with techniques to restore some hearing by bypassing the rigid middle ear bones with plastic or metal prostheses. However, more experience with these methods is still necessary to adequately evaluate them. In addition, an improved technique for vein graft closure of eardrum perforations has been developed, which may permit simpler, permanent repair of extensive eardrum damage. A surgical means for relieving the majority of severe cases of Meniere's disease was evaluated this year with considerable success.

Complex basic studies of auditory pathways have continued to progress during 1960. In one such project, investigators have utilized the electrical responses of single cells to noise to determine an integrative mechanism of cellular substations along certain nerve pathways.

EVALUATION OF THERAPY

As research in neurological and sensory disorders has advanced during the last decade, many new drugs for the treatment of these disorders have become available. Unfortunately, there has been no corresponding carefully planned evaluation of the results of the use of these drugs.

In an effort to clarify this entire area, the Institute last spring sponsored a symposium on the evaluation of drug therapy for the treatment of neurological and sensory disorders. In addition to a general session which considered methods of designing clinical trials for therapy evaluation, eight panels of well-known scientists considered the specific problems relating to particular disorders. These panels were on epilepsy, multiple sclerosis, vertigo, headache, diseases of the muscles, glaucoma, Parkinsonism and diseases of the basal ganglia, and neuropathies and neuralgias.

The panels are continuing their work and several of the groups have made considerable progress. In epilepsy, the panel has worked out a method of classification of cases and a preliminary form for use in a statistical analysis of results. The various drugs, of course, must be evaluated over a period of time with many patients to determine their effectiveness, toxicity, and total bodily effect in relation to each of the many types of epilepsy.

The panel on multiple sclerosis has a particularly difficult task because of the unpredictable course of the disorder. Frequent claims of beneficial results of therapy, often made on the basis of unsound data, lend urgency to the development of a sound and agreed-upon program for evaluation. The panel, therefore, is working to establish guidelines which will delineate rigid criteria for case selection and for the evaluation of improvement.

The problem of a proper evaluation of the drugs available for the various types of headaches is monumental. It is estimated that several hundred drugs have been put on the market in the last 25 or 30 years for migraine headache alone. One headache clinic is now testing 23 new drugs for migraine and has turned down many others. The remedies have increased ten-fold in recent years.

Here, too, problems of classification and evaluation are very difficult. Scientists in various areas of the country reporting results of therapy find their work often cannot be correlated with other research because of the lack of uniform procedures. Therefore, the panel on headaches has set three goals: the preparation of a uniform descriptive classification of types of headache; the preparation of a specific design for drug evaluation studies; and the discovery of specific chemical or physiological diagnostic criteria. The panel believes the first two goals can be achieved during 1961.

Over the next year it is anticipated that the activities of these several panels can have a major influence in bringing order to the present chaos of drug evaluation in relation to these and other important neurological disorders.

BROADENING THE BASE FOR NEUROLOGICAL RESEARCH

Increasingly, neurological research is coming to depend upon long-term projects requiring the use of complex equipment and involving a variety of technical skills. Such projects can evolve only upon a base of broad continuing support. As a participant in the new NIH program of clinical center

grants, the Institute is offering support to medical institutions (1) engaged in a multidisciplinary attack on specific diseases of importance in the neurological and sensory field or (2) engaged in a disciplinary attack on a group of neurological and sensory disorders. This support is for setting up patient beds and providing the salaries of key staff for such centers.

Under the clinical center grants program, we will be particularly interested in such specific problem areas as epilepsy, cerebral palsy, multiple sclerosis, cerebrovascular diseases, disorders of vision and hearing, and neurological diseases of infancy. Fourteen such grant applications have been received and will be reviewed at the next meeting of the Institute's Advisory Council.

Currently being considered is the possibility that this program eventually might provide a mechanism by which regional, multi-institutional clinical research centers could be developed. At such centers, outstanding investigators from medical institutions in a specific area could utilize necessarily complex and highly expensive facilities for research--facilities which would not be feasible for an individual institution.

Not a part of the clinical center program but of a similar nature is a broadscale project in neurophysiological research at the University of California at Los Angeles, supported by a large Institute grant. This project, being undertaken by the University's Brain Research Institute, will examine neurochemical and neuropharmacological phenomena in patients selected primarily from those suffering from Parkinsonism and other hyperkinetic disorders, intractable pain, mental disorder, epilepsy and protracted psychosomatic disorders. It is anticipated that the program will result in the development of improved methods of treatment, greater understanding of the nature of behavorial and medical disorders, and expanding insight into the mind-brain relationship.

INSTRUMENT DEVELOPMENT

Of particular concern in neurological research is the need for the development of highly specialized instruments. This year, two projects have received consideration. One relates to the development of a research center within which highly developed computers will be used for the analysis of the activity of the nervous system. Such computers will be essential to a further understanding of the complex interactions of neuronal pathways within the brain. The second is for a special microscope designed for the automatic counting and analysis of nerve cells seen in microscopic preparations of brain sections. Such projects which rely heavily upon the technical knowledge and specialized skills of the industrial scientists have much to offer to medical research.

TRAINING FOR NEUROLOGICAL RESEARCH

The life blood of research is the flow of young, well-trained investigators into its stream. No aspect of the Institute's program can outweigh

training in ultimate significance to the cure of disease. Although the total manpower involved in this program remains small, this year has again seen a healthy development in all areas.

During 1960, there were approximately 190 Institute-supported programs of research training in the clinical and laboratory aspects of the neurological, visual, and auditory sciences. New grants approved during the year were for seven new programs in neurological sciences, three new programs in clinical otolaryngology, and two new programs in clinical ophthalmology.

In the development of these new programs, special emphasis has been placed on the neglected field of pediatric neurology and on the basic sciences of neurochemistry, neuropharmacology, and neuropathology. Definite progress can be seen resulting from training in otolaryngology and related fields. Specifically, the number of applicants for special fellowships to further research training is increasing rapidly, and the number of graduates of training programs and the number of special fellows accepting fulltime academic appointments are increasing. Also noticeable is an increased number of trainees participating in original investigations--probably one of the most important developments to date.

One immediate major beneficial result from training grants in clinical ophthalmology has been the broadening of basic science training in this field. This leads not only to immediately increased research by members of the training staffs, but augurs well for the training of well-rounded researchers in the future.

During the year, the Institute has provided support for 84 post-doctoral fellows, and 213 special fellows. The latter program, which provides support for the advanced training of highly selected scientists has been particularly productive, with approximately 85 percent of the former trainees now occupying positions in research or teaching.

PROSPECTS FOR FUTURE RESEARCH

In March 1960, the Institute supported a conference sponsored by the Parkinson's Disease Foundation to review the status of research in Parkinsonism. This conference outlined two fundamental approaches to an understanding of the causes of this disorder: first, a detailed study of the microscopic changes in the brain of patients dying from Parkinson's disease--studies aimed at delineating with accuracy the structures and tissues affected by the disease process; and second, a more accurate knowledge of the chemical structure of those portions of the nervous system which are selectively destroyed by viruses or by specific chemical agents.

The approaches set forth by this conference exemplify the fundamental role which chemistry is assuming in relation to disorders of the nervous system. In the final analysis it is probable that the conquest of the major disease categories in neurology will depend to a large extent upon our ability to recognize and modify derangements of chemistry in relation to the nervous

system. For example, the discovery of a basic chemical defect has led to the development of an effective treatment for galactosemia and phenylketonuria. This year the demonstration that still another form of inborn error of metabolism may be influenced by dietary control has been shown.

There are a number of additional disorders affecting the nervous system more directly in which one must certainly give serious consideration to the probability of a basic chemical defect. The muscular dystrophies and familial periodic paralysis are associated with chemical abnormalities. Amyotrophic lateral sclerosis has a specific and symmetrical distribution which strongly suggests a chemical anomaly which should be capable of demonstration and control. Chemical abnormalities are evident in the epilepsies, and accurate knowledge of the nature of these anomalies will lead to more specific control than is now available. Important progress is being made in respect to the chemistry of cataract formation in the eye. The retinal degenerations are also a reflection of chemical defect.

With the recent rapid advances in chemical knowledge and technique, the application of this new knowledge to the study of neurological diseases offers a promising challenge. Through a general increase in the study of the fundamental chemistry of the nervous system, together with a broad-scale attack from many other disciplines, many new advances should be possible in the next few years.

Grants:		
Research	$31,152,000	
Fellowships	980,000	
Training	7,846,000	
Subtotal, grants		$39,978,000
Direct operations:		
Research	8,081,000	
Review and approval of grants	1,051,000	
Training activities	50,000	
Administration	306,000	
Subtotal, direct operations		9,488,000
Total 1962 appropriation request		$49,466,000

ANNUAL REPORT

Calendar Year - 1960

DIRECT TRAINING

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
NATIONAL INSTITUTES OF HEALTH

Funds under this activity ($50,000) provide for the support of the in-service training program of the Institute. This program makes it possible for the Institute to secure qualified staff for some of its operations by training younger scientists in particular skills necessary to carry on certain program operations.

In cooperation with the Communicable Disease Center in Atlanta, Georgia, the Institute is presently supporting training in the broad concepts of epidemiology and biometry. Basic training and experience in the field of the more acute and widespread communicable diseases offer an introduction to the more complex and protracted epidemiological and biometric problems of neurological and sensory disorders and chronic diseases.

Training is obtained in universities and other appropriate institutions in order to supplement already acquired experience and academic training where special needs exist in the total neurology research program.

Currently, the Institute is supporting one year of training for two professional staff members. Dr. Roscoe Brady is at the University of California, Berkeley, California, to investigate the detailed nature of the mechanism of vitamin B_{12} mediation reactions, in collaboration with personnel at the University, and to take a course on electronic mechanisms of organic reactions.

Dr. Mark Lane is at the Mayo Clinic, Rochester, Minnesota, to study a large number of varied neurological conditions with opportunities to observe varied diagnostic procedures and their interpretations in large numbers, as in neuroradiology, electroencephalography and neuropathology.

Short term courses, generally of a specific technical nature, are also undertaken in this activity. During the past year the Institute provided support for approximately 22 employees attending short term courses. Currently there are 12 employees attending courses at the U.S. Department of Agriculture Graduate School and Howard University to further their knowledge in their respective fields of research and statistics.

ANNUAL REPORT
Calendar Year, 1960
Extramural Programs Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

I RESEARCH GRANTS

1. Program Developments

Research in Neurological and Sensory Sciences continued to flourish during the year, aided by a substantial increase in the research grants budget appropriation for the fiscal year that started July 1, 1960. However, the appropriation figure of $38,797,700 for this activity is somewhat misleading, since several obligations against this budget were instituted to support other important activities of a central nature. So, after adjusting for reserves and transfers and unprogrammed funds, the actual working budget for research grants became $28,052,000. The budget for the previous fiscal year was $23,746,000; thus, it is apparent that the actual increase, effective July 1960, amounted to 18%.

A. Number of Grants: - In December 1960 the NINDB supported 1,284 active research projects. A year ago at this time there were 1,107 active grants. The net gain in numbers of active research grants on a year-to-year basis was 177, or 16%.

During the year 351 new projects were activated, using $7,156,669. The average new grant was in the amount of $20,400. 328 new applications were not approved.

Of the competing continuation applications, 144 were approved by the Council in an average amount of $19,100. 22 continuation applications were not approved.

Of the supplemental requests the Council approved 129 for an average of $18,200 and did not approve 39.

In summary, the Council this year recommended approval of 624 competing applications of all types, for a total of $12,274,360. This represented 51% of the requested funds and 62% of the numbers of applications. The year-to-year increase over last year's approvals is 170 in numbers (37.2% and $4,800,000 (60%)). (Note: It should be emphasized that the numbers of approvals by the Council during the year are not related in any simple way to the numbers of active research projects during the year, since each of the latter may represent two or more actions for approval, for supplements or continuations.)

B. Staff Organization: - The size of the Research Grants Program in neurologic and sensory disorders is now so large that a more formal organization of staff became a necessity. To further the mission of the Institute, it was decided to divide the total program into sub groups, along disease or categorical lines. These are as follows, with the dollar value, and percent of total budget as of July 1960:

(1)	Perinatal Collaborative Program	$4,631,000	20%
(2)	Metabolic and Degenerative Diseases (Mental deficiency, Multiple Sclerosis, Neuromuscular)	4,100,000	18
(3)	Vision Disorders	4,012,000	17
(4)	Involuntary Movements (Epilepsy, Parkinsonism, Ataxias)	3,050,000	13
(5)	Hearing and Speech Disorders	1,967,000	9
(6)	Autonomic, Peripheral, Infectious	1,800,000	8
(7)	Cerebrovascular Disease	1,337,000	6

The professional staff person in charge of each of these disease groups will assume responsibility for programming, evaluation of progress, and general administration along multidisciplinary lines.

C. Status of New Programs: - This year marks a turning point in the Extramural Programs of all Institutes, including Neurology; philosophy and guidelines were developed for several new programs, such as categorical clinical research centers, broad research program projects, institutions grants, categorical senior fellowships and career professorships. Beginnings were made in developing each of these new programs, although for the most part they will not be fully activated until next year.

A clinical research center grant was awarded by NINDB late last year, to the University of California, Los Angeles. The purpose of this project was the application of modern neurophysiology to neurologic disease. The grant is in the amount of $264,638 for the current period. In addition to this ongoing program, the NINDB continues to contribute $500,000 yearly to support the progress of general clinical centers, administered and awarded by the National Advisory Health Council.

D. Status of Ongoing Programs: -

Cerebrovascular disease: "Stroke" continues to be one of the major killers and cripplers in the country, yet our support of research into this important neurologic disease is the smallest of any of our programs ($1.34 millions, or 5.7% of the budget). In a major effort to expand interest, two actions have been taken during the year: First, an international congress on this disease was organized and held in Miami Beach in June, following the meeting of the American Academy of Neurology, which was the official sponsor. Progress reports were included on the two Cooperative studies supported by NINDB (i.e., aneurysm and anticoagulant evaluation study). In addition a grant was made to support the 3rd Princeton Conference on cerebrovascular disease which is scheduled for January 1961. Second, a

cerebrovascular advisory group was established by the Director, NINDB. This committee is made up of Irving Wright (Internist), Guy Odum (Neurosurgeon), George Margolis (Neuropathologist), C. Miller Fischer (Neurologist), Clark Millikan (Neurologist), A. D. Sahs (Neurologist) and James O'Leary (Neurologist). The purpose of this committee is to review the status of research in this disease and to advise and suggest on procedures to expand research. A brochure on "Outlines of Research in C. V. Disease" was prepared and widely distributed (350 copies).

In other areas, a decision was taken to initiate a new edition of "Classification of C. V. Disease" under Clark Millikan. The Cooperative Study on the use of anticoagulants in C.V. disease has apparently turned up negative conclusions, leading to a decision by the group to terminate its activities by September of 1961. The Cooperative Aneurysm study has yielded a wealth of data which have not yet been thoroughly examined. This group will continue its study for at least another year. The WFN international study on epidemiology and neuropathology of strokes has organized a protocol and procedure; presumably plans will be organized to undertake the major study starting next year.

Hearing Disorders: This program is continuing to grow rapidly. Of the 1960 budget, 7.0% (1.6 million) went to support studies in this area, as compared with 5.5% (0.9 million) the year previous. This is a 77% increase in dollar expenditure. The Council approved in principle a request by the Southern Regional Education Board for funds to hold a conference on "Hearing and Speech Pathology in the South".

Speech Disorders: Closely related with the other end of the communication line, speech studies are also rapidly increasing. The $334,000 this year was a $168,000 increase (90%) over last year. Special activity relates to a conference, approved in principle by the Council, for a "Workshop on Research Potentials in Voice Physiology", to be held at Syracuse University.

Vision Disorders: Research into vision was supported at a level of $4,012,000 this year; this was $1.16 million (or 40%) more than last year, although the numbers of active grants increased only 18% (to 236). In the vision field, the most active increase in interest occurred in the glaucoma problem; the number of new grants for glaucoma studies increased 44% to 46 grants (the funds to support this work increased 55% to $850,000). It is doubtful that this increase was stimulated by the Collaborative Glaucoma study in six institutions, since this has been slow in getting started, and has not yet put forth a progress report on its study to evaluate techniques for detecting glaucoma.

During the year, the Council agreed to support, in part, the IX International Congress on Ophthalmology, to be held in India in 1962.

General Neurologic Disorders: As in the past few years, about half of the research grant budget supported studies in the general area of neurology; the perinatal collaborative accounted for another 20%, and

research projects in all sensory areas received the remaining 30% of the funds.

Of general interest is a report given to the Council by Mr. George Payne on an analysis of characteristics of first-time grantees of the NINDB as compared with total NIH. He finds that the NINDB grantee was considerably younger than the average NIH grantee when he first obtained a research grant: 43% of the first-time NINDB grantees were under 34 years, as compared with 34% for all Institutes. More of the NINDB first grantees were connected with medical schools, primarily in the preclinical sciences. (39% of NINDB grantees as compared with 29% of NIH grantees). More Ph.Ds were among NINDB first-grantees (66%) than NIH first-grantees (61%), and many more also had the M.D. degree in addition to the Ph.D. (13% vs 5%). The implications of these facts are interesting, and may reflect a more active training program. Certainly, if the younger scientists maintain their present interest in neurobiology, it is apparent that within two decades research in this science will be a much larger portion of the total biomedical research than it now is.

Related to this study of the first-time grantee, it is of interest to observe the general distribution of NINDB research grants. In Table 2, it is apparent that more than half our grants to medical schools go to preclinical departments, and that anatomy departments appear to be highly favored. Classical biochemists have not yet become intrigued by the nervous system as is evident from the relatively small support put into these departments.

Infectious Diseases: The total number of grants (13 for $270,000) in the Infectious Disease category is insignificant.

During the past two years there has been increased activity insofar as parasitic and infectious diseases are concerned. Some universities are starting study centers in Latin-American countries; one veterinary school has begun large-scale investigations of the Zoonoses, persons studying neurotropic viruses have been organizing their efforts, especially after outbreaks of human cases of encephalitis in parts of eastern U.S.A. where only cases in horses and birds had been reported heretofore. There have been WHO meetings of a "Study Group on Arthropod-borne Viruses" in September. The working papers indicate their concern over the lack of knowledge of the pathogenicity of many of these viruses. The WHO has been anxious to see more work done with the filarid disease, onchocerciasis, an important cause of economic blindness in some tropical areas of Central and South America and of study and control. It is contemplated that requests for grant support in these fields will be on the increase.

2. Research Developments

A. Conferences: - Several important international congresses are scheduled for the near future. The Council has considered requests for support and grants have been made to assist the 2nd International Congress of Neurosurgery (Washington); the International Congress of Neurology (Rome);

the International Congress of Neuropathology (Munich).

In addition, the Council has approved a grant of $75,000 to a Travel Selection Committee to financially assist younger scientists traveling in Europe to attend one or more of these meetings.

Other conferences held during the past year include:

(1) "International Symposium on Response of the Nervous System to Ionizing Radiation" (September 1960 - Northwestern University). Sponsored jointly with the AEC.

(2) "Opportunities for Research in Chronic Disease Hospitals". Bethesda, March 1960. This was part of a general program for encouraging research in custodial hospitals, where an abundance of long term clinical material is found.

(3) "Sumposium on Drug Therapy in Neurologic and Sensory Disorders". (Madison, May 1960). A follow up meeting was held in Chicago in September. This is part of the Council's program for exploring the status of drug evaluation and finding techniques and procedures for more accurately testing new drugs that may be proposed. This will be an ongoing program; the eight disease panels that were originally set up to develop in depth, will continue to function during the next year, aided by a grant for support of their activities.

(4) "Current Status of Parkinson's Disease" (New York, May 1960) sponsored jointly with the Parkinson Disease Foundation.

(5) "Abnormal Nervous Functions - Progress in Neurobiology Series-V" (Boston, August 1960).

B. Publications: - In 1960 reprints 835 papers published in scientific journals were placed on file by NINDB grantees. These were supported by 365 research grants.

C. Contributions from Specific Awards: - Dr. Nachmansohn (Columbia University) has published data that is said to explain the mechanism of local anesthesia; the anesthetic agents have been shown to act as anti-metabolites for the acetylcholine nerve impulse activating system. This will permit a much better prediction of action for any agent proposed in the future. Meanwhile, Dr. Geoffrey Bourne (Emory University) has published a note in Nature showing that the presumed artefacts seen in electron microscopic studies of the myelin sheath, are in fact hexagonal chambers, each abutting directly on the surface of the axon; presumably each is a little chemical factory in which the sodium exchange process takes place to propogate the impulse.

The American Academy of Ophthalmology and Otolaryngology's Sub-committee on Hearing in Children has produced preliminary results of significance and shows promise of further findings of fundamental importance.

The preliminary findings and data on acoustic environment and audiometer performance have been presented to two of the interested professional organizations (American Academy of Ophthalmology and Otolaryngology and American Speech and Hearing Association). Publication of the results is expected in the near future. Included in the materials are data related to hearing and hearing loss on approximately 4,000 children. An interesting observation has been that "the majority of otoscopically 'abnormal' ears (over 80%) as defined in this study do not show hearing loss, and conversely, many ears with hearing loss showed no observable otological abnormality. It has been demonstrated that audiometric testing alone cannot identify physical abnormalities of the ear, which may have predictive value in indicating hearing loss".

In recent years, while there has been a tremendous impetus given to the study of virus diseases, especially neurotropic ones like poliomyelitis, the encephalitides, and the tropical arthropod-borne virus diseases, relatively little investigation has been made of the sequelae. Most of the research has been of a serological or immunological nature. However, there are a few exceptions. Knox Finley's group in California has continued to make observations on after-effects of Western equine and St. Louis encephalitis in California, especially in regard to children. Sidney Grossberg and Robert J. Keehn, other NINDB grantees, have been studying the sequelae to Japanese encephalitis in former U. S. military personnel. These investigators have been able to secure large numbers of authenticated cases and controls and to secure follow-up examinations. In preliminary studies, using health questionnaires, it was found that the JE group had twice the proportion with neuropsychiatric complaints and about twice the percentage of diagnoses associated with organic neurologic disease, as compared to the controls. This data has not yet been written up for publication since it is awaiting clinical reexamination of patients.

There has been recent evidence that non-neurotropic virus diseases may be associated with neurological disease. British physicians have expressed some concern regarding Parkinsonism appearing with, or following Coxsackie infection (Ciba Symposium, September 1960, London). Some of the participants at the Montreal Conference on Parkinsonism (October 1959) believed that there were endemic cases of von Economo encephalitis as well as Parkinsonism resulting from Asian influenza. Since little is known about the etiology of Parkinsonism in general, it was felt that attention should be given to types of encephalitis as a possible source.

Some of the work being carried on in this field concerning influence of the nerve in limb regeneration is exemplified by investigations of two of our grantees. Dr. Charles Thornton has demonstrated that the apical cap (several layers of cells that stretch across the wound epithelium) is necessary for limb regeneration in that it directly influences the aggregation and outgrowth of the blastemal cells. He has suggested that the brachial nerves and the apical cap cooperate in controlling limb regeneration. When a limb is denervated, no apical cap is formed on subsequent amputation. Thornton has just shown that in a deafferent limb stump in which hyper-regeneration of motor nerves was induced so that limb regeneration will proceed typically, an apical cap forms without any evidence of nerve fibers in it.

This has been checked with the electron-microscope. Thus, nerves are needed for an apical cap to form but they need not be physically present in the cap -- only in the limb stump. Future work must decide whether the nerves act by way of the apical cap.

At the University of California, Drs. W. R. Adey, C. W. Dunlop, C. E. Henrix have conducted neurophysiological studies which suggest that the hippocampus may be the subcortical center of the brain responsible for the "selecting" experiences to be remembered, and for recalling the remembered experience. Related studies of the afferent and efferent pathways of the hippocampus and adjacent structures serve to confirm the study.

Drs. Bruce L. Ralston and Christos A. Papatheodoran have shown by means of direct cortical recordings that a characteristic pattern of brain wave activity precedes the development of an epileptic seizure. This electro-encephalographic pattern, unlike the spike patterns, is sharply localized and may, therefore, prove very useful in localizing epileptic lesions for neurosurgical extirpation.

In an important new approach to demyelinating disorders, Drs. Murray Bornstein and Stanley Appel at Mt. Sinai Hospital, New York, have discovered that nerve demyelinization can be induced and studied in tissue culture. The demyelinization has been produced by treatment with specific immune serum which is toxic only to brain tissue.

An effective method for suppressing allergic encephalomyelitis in guinea pigs has been reported. Encephalomyelitis is induced by injection of an encephalitogenic vaccine and subsequently suppressed by the administration of an active brain abstract. This important discovery is significantly related to the post-infectious and post-vaccinal encephalomyelitides and to multiple sclerosis. These findings have been reported by Drs. Chang-Mei Shaw, W. J. Fahlberg, and E. C. Alvord at Baylor University, and Dr. Marian Kies at NIMH.

Drs. E. A. Spiegel, H. T. Wycis, B. Rusy, and H. W. Baird of Temple University Medical Center have studied the meseucephalic tegmentum in patients with Parkinsonism. They have shown that the Parkinsonism tremor can be influenced from an area distant from the pyramidal system without impairment of voluntary movements. These findings will influence the neurosurgical ablation techniques now being used therapeutically in Parkinsonism.

Electron microscopic examination of swollen brain tissues in both mice and humans reveals that excess fluid accumulates within certain cells of the cerebrum, rather than in spaces between cells as has been previously theorized. These research findings suggest that cells may be responsible for the essential process of cerebral fluid transport. The findings have been described by Drs. Torack, Terry, and Zimmerman at Montefiore Hospital.

3. Major Problems

On October 14, 1960, the Chief of the Extramural Programs Branch

(Dr. Gordon Seger) resigned. This created problems. Activities continued under interim conditions through the November Council meeting and the rest of the year. Earlier, the Surgeon General had approved a reorganization plan which, in part, would have created a Research Grants Branch, and a Training and Fellowship Branch, each headed by a Chief, under an NINDB Associate Director for Extramural Affairs. This plan has not been put into effect because of staffing problems.

4. Plans for the Future

With the high interest in the new programs developing at the NIH, such as Center Grants, Program Grants, Institutional Grants, Career Professors, etc., it is sometimes forgotten that the basis for the grants program is still the individual project, and will remain so for at least the next few years.

Therefore, while laying plans to put the new programs on a solid foundation, and to recruit the best men and institutions to develop them, plans will also be expanded to put funds into the support of good research projects of interest to the program missions of the Institute. The purpose of the program is, after all, to further the solution of the cause, cure and management of neurological and sensory disorders.

5. Staff

Edwin L. Hove, Ph.D., Head, Research Grant Section *

Dwight C. Monnier, Ed.D. (On detail from June 24, 1960)

Herbert T. Dalmat, Ph.D. (On duty from August 14, 1960)

Kathryn R. Lewis, M. D. (On duty from September 6, 1960)

Murray Goldstein, D. O. (On duty from July 1, 1960)

Frederick Gutter (Detailed to Training Section August 1, 1960)

* Assistant Chief, Extramural Programs Branch and
Acting Chief, Extramural Programs Branch beginning October 14, 1960

TABLE I

NUMBERS OF RESEARCH GRANT ACTIONS-1960

(In thousands, with numbers in parenthesis)

Council	New		Competing Continuations		Supplements		Totals	
	Requests	Approval	Requests	Approval	Requests	Approval	Requests	Approval
March 1960	$5,447 (211)	$2,573 (114)	$1,339 (72)	$1,042 (59)	$1,195 (58)	$ 823 (48)	$7,981 (341)	$4,439 (221)
June 1960	4,649 (232)	1,957 (116)	880 (46)	762 (44)	845 (47)	429 (36)	6,375 (325)	3,149 (196)
November 1960	6,878 (236)	2,625 (121)	1,261 (48)	953 (41)	1,741 (63)	1,106 (45)	9,881 (347)	4,685 (207)
Totals	$16,975 (679)	$7,156 (351)	$3,481 (166)	$2,758 (144)	$3,781 (168)	$2,359 (129)	$24,238 (1,013)	$12,274 (624)
Approval Rates 1. Dollars		42.1		70.5		63.5		51.0
2. Numbers		51.8		86.5		77.0		62.0

TABLE II

1960 RESEARCH GRANTS - NINDB

Distribution by Type of Institution with Breakdown by Departments in Medical Schools

Total Research Grant Appropriation ---------- $23,662,068

Total 1960 Research Grant Funds Awarded to:

(1)	Medical Schools ----------	$12,111,712	(51.2%)
(2)	Hospitals ----------	3,086,082	(13.0)
(3)	Universities (Arts & Sciences) ---------	2,177,448	(9.2)
(4)	Perinatal Collaborative Programs -------	4,631,665	(19.6)
(5)	Others ----------	1,655,161	(7.0)

MEDICAL SCHOOLS

Department	No. of Grants	Amount	% of Total Grants to Medical Schools	% of Total Research Grants Appropriation
Pre-Clinical				
Anatomy	132	$1,911,370	15.8%	8.1%
Physiology	84	1,519,153	12.5	6.4
Pharmacology	72	1,278,090	10.6	5.4
Pathology	38	638,807	5.3	2.7
Biochemistry	22	424,843	3.5	1.8
Others	11	135,240	1.1	0.6
Totals	359	$5,907,503	48.8%	25.0%
Clinical				
Ophthalmology	88	$1,578,498	13.0%	6.7%
Neurology	68	1,087,851	9.0	4.6
Surgery	43	779,363	6.4	3.3
Neurosurgery	35	681,330	5.6	2.9
Medicine	39	611,014	5.0	2.6
Pediatrics	30	598,330	4.9	2.5
Otolaryngology	27	458,256	3.8	1.9
Others	26	409,567	3.4	1.7
Totals	356	$6,204,209	51.1%	26.2%

1960 ANNUAL REPORT
of the
National Institute of Neurological
Diseases and Blindness

II TRAINING GRANTS

A. Neurological Sciences Research Training

1. Program Developments and Accomplishments

One hundred and thirteen programs of research training in clinical and laboratory aspects of the neurological sciences were in effect during 1960 with a total dollar value of $4,797,533, an increase of $1,828,434 over the previous year. The monetary increase is accounted for not only by the activation of new programs and larger numbers of trainees participating in all programs, but also by the extra funds allocated for adjusting the beginning dates of training grants to July 1 to coincide with the academic year. A large number of programs were so adjusted, and it is expected that the remainder will be completed during fiscal year 1961.

Nine programs are scheduled for termination, six in the clinical and three in the basic science area. Lack of progress in developing research-orientation in some clinical training programs, failure to attract trainees, and loss of program directors either by retirement or moving to another institution were reasons for discontinuance of these programs.

Seven new programs (four in Clinical Neurology, one in Pediatric Neurology, one in Neurophysiology, and one in Neurosurgery) were approved with a total dollar value of $218,464.

The programs in Clinical Neurology and its subdivisions now being supported are as follows:

Clinical Neurology	64	$3,094,464
Pediatric Neurology	10	146,305
Neurosurgery	2	11,966
Neuroradiology	2	50,115
	78	$3,302,850

B. Basic Sciences

Included in the total given above for the Neurological Sciences are 35 programs in the basic neurological sciences being supported at the level of $1,444,683. These are listed below by field.

Neuroanatomy	5	$ 201,197
Neurochemistry	4	214,824
Neuropathology	15	408,652

Neuropharmacology	4	166,722
Neurophysiology	7	453,288
	35	$1,444,683

1. Neurosurgery

Since the meeting of the "ad hoc" Committee on grant support of training in Neurological Surgery, which was held in Bethesda on February 24, 1958, there has been increasing demand from neurosurgeons for NINDB to support postgraduate research training in this field. In its report dated March 1, 1958, the ad hoc Committee had recommended that the subject of training in neurological surgery be reviewed and re-evaluated after a period of two years. Accordingly a second ad hoc Committee of prominent neurosurgeons, which included some members of the earlier group, was convened in Bethesda on March 26, 1960. This group concluded that although no encouragement was needed to induce students to enter clinical neurosurgery training, the time was now ripe to initiate support of advanced training for research in order to increase the supply of investigators in the field of neurological surgery. At its meeting in June 1960, the National Advisory Neurological Diseases and Blindness Council approved the recommendation of the committee to support training in neurosurgery with grants, in accordance with the general policies and procedures now in effect applying to NINDB training grants. The Council did not accept a recommendation by the conferees that a separate training committee be set up to review neurosurgery training grant applications. One grant has been approved, and review of several more in this area is pending.

2. Teacher-Investigator Development Grants

Several applications were received as a result of the inauguration of Teacher-Investigator Development Grants, which are designed to support a full-time teacher-investigator in institutions not at present providing its students this type of training in the neurosensory field. However, as yet no grant has been recommended for approval, since the few candidates proposed were not regarded as of sufficiently high caliber to fulfill the purpose of these grants. It is believed that as the program purposes become better understood, there will be increased numbers of suitable candidates to whom such awards may be made. Many of the persons now about to complete training under NINDB training programs, either as direct or indirect trainees, should by this time be qualified for candidacy for Teacher-Investigator Development Grants.

3. Contributions Resulting from Particular Awards

As pointed out in reports of previous years, it is as yet too early to attempt to evaluate these programs in terms of significant research accomplishments by trainees. Nevertheless, the increase both in numbers of programs of high quality, as well as the increased number of trainees who are being attracted to such programs, indicates that the time is not far off when we should have an accumulation of

data which will make an objective evaluation possible.

4. Major Problems Encountered

Medical research as a specialized type of scientific research is an activity thought of commonly as a mechanism for solving problems such as the prevention and cure of disease, but medical research is also an activity which produces new knowledge. As new facts are uncovered, new questions arise which require new answers in the form of new facts and interpretations.

This type of chain-reacting activity needs a continuous supply of researchers whose task it becomes not only to perform research but to train new investigators.

The major problem which arises in the training area is recruiting potential teacher-investigators for academic careers and providing full time academic career opportunities for those who have completed their training.

With the improvement of facilities and other inducements such as provision for more adequate salaries, it should be possible to compete more successfully for potential trainees now being attracted to practice or more lucrative research areas.

Lastly, the Teacher-Investigator Development Grants, the newly created Career Research Professorship Grants and the establishment of Clinical Research Centers should help in providing increased opportunities for larger numbers of new researchers.

5. Changes in the Program

During the past year it has become apparent that the increased volume of work would require a change of committee organization to handle the larger load. The undesirable alternative was to lengthen the time of each meeting and to increase the size of the committee to unwieldy proportions. In meeting this problem, it was decided not only to fill the vacancies left by committee members completing their terms, but also to add members to a total of 26 and to divide the Committee into two sections (A and B), each with its own Chairman and Executive Secretary. The Chairman of each section would serve as associate chairman of the other, thus providing for some degree of integration. Dr. Aura Severinghaus, Associate Dean, College of Physicians & Surgeons, Columbia University, is Chairman of Section "A", with Mr. Morris C. Leikind as Executive Secretary. Dr. Joe Brown, Head, Neurological Section, Mayo Clinic is Chairman of Section "B", with Dr. Bernard Shacter designated as Executive Secretary. At the same time, to reflect more accurately the increased emphasis on research training and the broadened responsibilities of the committee, the name of the Neurology Postgraduate Training Committee has been changed to Neurological Science Research Training Committee. These

changes were effective on October 1, 1960.

The following persons completed their terms on September 30, 1960:

Dr. Adolph L. Sahs, Iowa State University,
Iowa City, Iowa

Dr. Gilbert H. Glaser, Yale University
School of Medicine,
New Haven, Connecticut

Dr. Henry G. Schwartz, Washington University
St. Louis, Missouri

Dr. Charles Van Buskirk, University of Maryland
School of Medicine
Baltimore, Maryland

Dr. Abner Wolf, College of Physicians
and Surgeons, Columbia University
New York, New York

New members added to the committees for a four-year term effective October 1, 1960, are:

Dr. J. Gordon Millichap, Mayo Clinic
Rochester, Minnesota

Dr. Donald D. Matson, Harvard University
Medical School
Boston, Massachusetts

Dr. Jerzy Rose, University of Wisconsin
Medical School
Madison, Wisconsin

Dr. Mannie M. Schechter, Albert Einstein
College of Medicine
Yeshiva University
New York, New York

Dr. James A. Bain, Emory University
Atlanta, Georgia

Dr. Edwin B. Boldrey, University of California
School of Medicine
San Francisco, California

Dr. Joseph Foley, Seton Hall College of
Medicine and Dentistry,
Jersey City, New Jersey

Dr. George Margolis
Medical College of Virginia
Richmond, Virginia

Dr. Juan M. Taveras
College of Physicians and Surgeons
Columbia University
New York, New York

Dr. Aura E. Severinghaus (term extended for 2 years)
College of Physicians and Surgeons
Columbia University
New York, New York

The list of members of the newly constituted sections of the Neurological Science Research Training Committee is appended below:

NEUROLOGICAL SCIENCE RESEARCH TRAINING COMMITTEE

SECTION "A"

Aura E. Severinghaus, Ph.D. (Chairman)
Associate Dean (1960-62)
College of Physicians and Surgeons
Columbia University, New York

Joe R. Brown, M.D. (Assoc. Chairman)
Head, Neurological Section
Mayo Clinic
Rochester, Minnesota

Orville T. Bailey, M.D. (1959-63)
Professor of Neurology
Psychiatric Institute
University of Illinois
College of Medicine

Raymond N. Bieter, M.D. (1957-61)
Professor and Head
Department of Pharmacology
Medical School
University of Minnesota
Minneapolis, Minnesota

J. Gordon Millichap, M.D. (1960-64)
Pediatric Neurologist
Division of Pediatrics
Mayo Clinic, Rochester, New York

Donald D. Matson, M.D. (1960-64)
Associate Clinical Professor
of Surgery
Harvard University Medical School
Boston, Massachusetts

Martin G. Netsky, M.D. (1959-63)
Professor of Neurology and
Neuropathology
Wake Forest College
The Bowman Gray School of Medicine
Winston-Salem, North Carolina

Fred Plum, M.D. (1959-63)
Associate Professor of Neurology
University of Washington
Medical School
Seattle, Washington

NEUROLOGICAL SCIENCE RESEARCH TRAINING COMMITTEE

SECTION "A" (Cont'd.)

Thomas W. Farmer, M.D. (1959-63)
Professor of Neurology
School of Medicine
The University of North Carolina

Joseph C. Hinsey, Ph.D. (1959-63)
Director, New York Hospital
Cornell Medical Center

Jerzy Edwin Rose, M.D. (1960-64)
Professor of Physiology
University of Wisconsin
Medical School

Roger J. Rossiter, B.M., B.Ch. (1960-63)
Department of Biochemistry
University of Western Ontario
London, Ontario, Canada

Mannie M. Schechter, M.D. (1960-64)
Associate Professor of Radiology
Albert Einstein College of Medicine
Yeshiva University

EX OFFICIO
Morris C. Leikind, Executive Secretary

NEUROLOGICAL SCIENCE RESEARCH TRAINING COMMITTEE

SECTION "B"

Joe R. Brown, M.D. (Chairman) (1957-61)
Head, Neurological Section
Mayo Clinic
Rochester, Minnesota

Aura E. Severinghaus, Ph.D. (Associate Chairman) (1960-62)
Associate Dean
College of Physicians and Surgeons
Columbia University
New York 32, New York

Vahe E. Amassian, M.D. (1959-62)
Professor of Physiology
Albert Einstein College of Medicine
Yeshiva University
New York, New York

Maynard Cohen, M.D. (1959-63)
Professor of Neurology
University of Minnesota Medical School
Minneapolis, Minnesota

Philip R. Dodge, M.D. (1958-62)
Associate Neurologist
Massachusetts General Hospital
Boston, Massachusetts

Joseph Michael Foley, M.D. (1960-64)
Professor of Neurology
Seton Hall College of Medicine and Dentistry
Jersey City, New Jersey

NEUROLOGICAL SCIENCE RESEARCH TRAINING COMMITTEE

SECTION "B" (CONT'D)

David Bodian, M.D. (1958-62)
Professor and Director
Department of Anatomy
Johns Hopkins University
School of Medicine
Baltimore, Maryland

Edwin B. Boldrey, M.D. (1960-64)
Associate Professor of
Neurological Surgery
University of California
School of Medicine
San Francisco, California

David Nachmansohn, M.D. (1958-62)
Professor of Biochemistry
College of Physicians and Surgeons
Columbia University
New York, New York

George Margolis, M.D. (1960-64)
Professor of Pathology
Medical College of Virginia
Richmond, Virginia

George E. Miller, M.D. (1959-63)
Director of Research in Medical
Education
University of Illinois College of
Medicine
Chicago 12, Illinois

James W. Stephens, M.D. (1957-61)
Associate Professor of Neurology
University of Colorado Medical
Center
Denver, Colorado

Juan M. Taveras, M.D. (1960-64)
Professor of Radiology
College of Physicians and Surgeons
Columbia University
New York, New York

EX OFFICIO
Bernard Shacter, Ph.D., Executive Secretary

6. Program Objectives for 1961

Although the number of training programs in Clinical Neurology will probably continue at present levels, much greater emphasis will be placed upon the research orientation of such programs than in the past. Thus the research activities of the program directors and their departments will come under more detailed scrutiny to evaluate their role in the development of broad-gauge medical scientists who can conduct independent investigations and are also qualified as educational leaders, to attract, inspire and train succeeding generations of scientists. At the same time, efforts will be made to increase the number of programs in Pediatric Neurology, Neuroradiology and Neurosurgery since the need for trained teachers and investigators in these areas is still critical.

It is, however, in the broad field of the basic sciences of neurology (i.e., neuroanatomy, neurochemistry, neuropathology, neuropharmacology and neurophysiology) that the most extensive efforts

will be made to increase the number of new programs. Not only is the need for more investigators most critical in these areas but the potential for scientific advances is greatest. The continuing increase in medical research funds voted by the Congress, serve only to emphasize the needs for more trained manpower of the highest quality to make effective use of these financial resources. Programming in the area of the neurological sciences is a complex educational operation. In addition to acquainting the scientific community with the training opportunities, the facilities available, and the objectives to be attained, efforts must be directed toward earlier recruitment of prospective research trainees. With the demands for trained scientific manpower increasing faster than workers can be trained, lengthening of a worker's productive life through earlier recruitment into a given field becomes a significant means of alleviating shortages.

7. Staff Assignment

Mr. Morris C. Leikind - Executive Secretary
Mrs. Elizabeth C. Hartman - Acting Head, Training Section
Extramural Programs Branch

8. Summary of Council Actions

Neurological Science Training Grant Applications -
Calendar year 1960.

NEUROLOGICAL SCIENCES

Area & Type	March '60 Council Requested	Approved	June '60 Council Requested	Approved	Nov. '60 Council Requested	Approved
Clin. Neuro.						
New	$191,266 (4)	$111,626 (3)	$ 43,193 (2)	$ 14,800 (1)	$ 68,124 (2)	---
Cont.	237,553 (6)	225,913 (6)	292,523 (6)	136,098 (3)	161,455 (2)	---
Suppl.	181,733 (8)	164,253 (6)	81,013 (5)	39,147 (3)	29,531 (2)	$ 12,755 (2)
TOTAL	$610,552	$501,792	$416,729	$189,945	$259,110	$ 12,755
Ped. Neuro.						
New	---	---	$ 32,808 (1)	$ 22,808 (1)	---	---
Cont.	$ 16,632 (1)	$ 16,632 (1)	21,523 (1)	21,523 (1)	---	---
Suppl.	---	---	---	---	$ 2,916 (1)	$ 2,052 (1)
TOTAL	$ 16,632	$ 16,632	$ 54,331	$ 44,331	$ 2,916	$ 2,052
Neurosurgery						
New	---	---	---	---	$ 90,426 (3)	$ 8,078 (1)
Cont.	---	---	---	---	---	---
Suppl.	---	---	---	---	---	---
TOTAL	---	---	---	---	$ 90,426	$ 8,078
Neuroradiology						
New	$ 35,950 (1)	---	---	---	---	---
Cont.	---	---	---	---	---	---
Suppl.	---	---	---	---	---	---
TOTAL	$ 35,950	---	---	---	---	---
Neuroanatomy						
New	---	---	---	---	---	---
Cont.	---	---	---	---	---	---
Suppl.	---	---	$ 23,200 (1)	$ 23,200 (1)	---	---
TOTAL	---	---	$ 23,200	$ 23,200	---	---

NEUROLOGICAL SCIENCES (Cont'd.)

Area & Type	March '60 Council Requested	Approved	June '60 Council Requested	Approved	Nov. '60 Council Requested	Approved
Neurochemistry	$					
New	---	---	$ 13,230 (1)	---	---	---
Cont.	---	---	---	---	$ 55,728 (1)	$ 55,728 (1)
Suppl.	---	---	---	---	---	---
TOTAL	---	---	$ 13,230	---	$ 55,728	$ 55,728
Neuropathology						
New	$ 12,690 (1)	$ 12,690 (10)	$ 30,840 (1)	$ 30,792 (1)	$ 38,890 (1)	---
Cont.	61,755 (3)	58,703 (3)	40,364 (2)	40,364 (2)	---	---
Suppl.	1,452 (1)	1,452 (1)	2,237 (1)	2,237 (1)	47,736 (1)	$ 44,600 (1)
TOTAL	$ 75,897	$ 72,845	$ 73,441	$ 73,393	$ 86,626	$ 44,600
Neuropharmacology						
New	---	---	---	---	$103,044 (2)	$ 32,400 (1)
Cont.	---	---	---	---	---	---
Suppl.	---	---	---	---	---	---
TOTAL	---	---	---	---	$103,044	$ 32,400
Neurophysiology						
New	$ 30,265 (1)	---	$ 67,992 (1)	$ 67,992 (1)	---	---
Cont.	---	---	---	---	$ 85,820 (2)	$ 78,820 (2)
Suppl.	6,592 (1)	$ 6,592 (1)	$ 3,150 (1)	$ 3,150 (1)	---	---
TOTAL	$ 36,857	$ 6,592	$ 71,142	$ 71,142	$ 85,820	$ 78,820
GRAND TOTAL	$775,888	$597,861	$638,843	$402,011	$683,670	$241,433

B. Communicative Disorder Research Training

1. Program Developments

At the beginning of 1960 there were 25 training programs in Clinical Otolaryngology, two in Medical Audiology, and three in the Sensory Physiology of hearing and speech, a total of 30, financed in the amount of $613,544. One grant in Sensory Physiology embraces jointly hearing and vision and is administered by the Vision Research Training Committee.

There were 143 trainees enrolled in these programs. During the year, four of the grants in Clinical Otolaryngology were disapproved for continuation because the Council believed insufficient progress had been made toward developing teachers and investigators; however, one of these institutions has applied for a Teacher-Investigator Development Grant, and two others have made considerable improvement in their programs and are planning to resubmit applications for training support.

Three new programs in Clinical Otolaryngology were initiated in 1960, along with two in Medical Audiology and one in Sensory Physiology. Thus, at the end of the year there were 28 programs in Clinical Otolaryngology (including the four grants scheduled for termination June 30, 1961), four in Medical Audiology, and four in Sensory Physiology of Speech and Hearing (excluding the joint Otolaryngology and Ophthalmology grant). These programs embraced 194 trainees, and were financed in the amount of $874,225. This figure includes extra funds used to adjust the beginning date of some grants to July 1.

Particularly significant is the progress being made in the basic sciences. Many of the training programs in Medical Audiology and Sensory Physiology have a full complement of trainees and are receiving many inquiries from prospective trainees. The directors of the programs are acutely aware of the limited number of institutions qualified to give this kind of training and are extremely anxious to expand the existing programs, thereby meeting the national needs in this area.

2. Contributions Resulting from Particular Awards

Definite progress can now be seen resulting from training in Otolaryngology and related fields. Specifically, the number of applicants for Special Fellowships to support further research training is increasing rapidly, and the number of graduates of training programs and/or the number of Special Fellows accepting full time academic appointments is increasing. Also noticeable is an increased number of trainees participating in original investigations; this is probably one of the most important developments to date.

3. Major Problems Encountered

A major problem continues to be that of inducing trainees to stay in academic medicine after completion of training. There remains a vast monetary differential between academic otolaryngology and private practice. However, two factors seem to be affecting a trend toward full time research and teaching as a career objective, namely, the growing national interest in investigative pursuits and the increasing awareness of medical schools of the value of full time paid staff persons. With this in mind, and remembering the expansion of existing medical schools and the proposed building of new schools, it is necessary to expand and improve existing training programs and to establish new programs with definite research orientation. This statement is especially true in regard to the basic sciences relating to hearing and speech.

4. Changes in the Program

A. Dr. Shacter served as Executive Secretary both to the Ophthalmology and Otolaryngology Postgraduate Training committees until July 1960. At this time, Dr. James R. Leonard assumed the position of Executive Secretary of the Otolaryngology Committee, and Dr. Shacter devoted his interests to the Ophthalmology Committee and one section of the Neurology Training Committee.

B. The following members of the Otolaryngology Committee completed their terms with the September meeting:

John E. Bordley, M. D.
Director, Department of Otolaryngology
The Johns Hopkins Hospital
Baltimore, Maryland

Dean M. Lierle, M. D.
Professor and Head
Department of Otolaryngology
State University of Iowa
Iowa City, Iowa

George F. Reed, M. D.
Assistant Otolaryngologist
Massachusetts Eye and Ear Infirmary
Boston, Massachusetts

C. The name of the Committee was changed to Communicative Disorder Research Training Committee on October 1, 1960. This name reflects more accurately the broadened responsibilities of the Committee.

D. New members accepting appointments on the Communicative Disorder Research Training Committee for a four-year term effective October 1, 1960, are:

Merle Lawrence, Ph.D.
Physiological Acoustics Laboratory
University of Michigan
Ann Arbor, Michigan

William G. Hardy, Ph.D.
Director, Hearing and Speech Center
Associate Professor of Otolaryngology
and Environmental Medicine
The Johns Hopkins Hospital
Baltimore, Maryland

5. Program Objectives for 1961

A. To re-emphasize the research orientation of trainees in both clinical and basic science training programs. Although Program Directors seem to be aware of the primary objective of the National Institute of Neurological Diseases and Blindness support of training programs, and there is a definite trend in the desired direction, it is hoped that momentum will be added to the movement.

B. Dissemination of information to potential trainees regarding governmental support available, academic and research positions available, and other pertinent data. It has become clear that many highly-motivated individuals complete their training at good institutions and drift into private practice without being aware of the opportunities in the academic world.

C. An Otolaryngology Training Conference is to be held at the National Institutes of Health in the spring of 1961. The primary objective of this Conference is the attempt to formulate ways of improving training, research and clinical, in Otolaryngology and related fields. It is planned to invite all Program Directors, some Special Fellows with suitable qualifications, and most of the top scientists in the country who have an interest in otolaryngological training. The Conference is being held prior to the International Congress on Otolaryngology, which meets every four years. The Congress will be held in Paris in July 1961, and a section on training is planned.

D. To continue the evaluation of program needs. It is recognized that research in otolaryngology, and indeed in hearing and speech in general, constitutes only a small segment of the over-all research needs in biology and medicine. It is proposed to continue the evaluation of the needs in this area, and to adjust the development of the programs accordingly.

6. Staff Assignment

James Leonard, M. D.
Executive Secretary
Communicative Disorder Research
Training Committee

Elizabeth C. Hartman
Acting Head, Training
Section
Extramural Programs

C. Vision Research Training

1. Program Developments

At the beginning of the calendar year 1960, there were 35 active training grants in clinical ophthalmology. Of these, five were scheduled for termination, either because continuation had not been requested or the continuation application had been disapproved. During the year, applications were received for the initiation of six new training grants, of which two were approved and activated on July 1. On the other hand, two of the nine active grants competing for continuation were disapproved. In addition, continuation was not requested for one active grant with a 1960 expiration date. Thus, at the end of the year there were 33 active programs, of which four were scheduled for termination. The net change over the year, therefore, was the loss of one viable grant.

As shown in Table 1, the approval rate of clinical ophthalmology grants considered by the Council in 1960 approximated that for 1959. There was again an upward trend in the average size of individual grants. This was evidenced by the increased amounts which were approved for continuation grants, and by the supplemental funds awarded to four grantees.

Total grant support in clinical ophthalmology at the end of the year was approximately $1,693,312. This amount includes funds used to adjust some of the programs to a July 1 period beginning date.

In basic science areas there were two grants in effect at the beginning of the year, one for overall training in sensory physiology, including visual physiology at the University of Michigan, and one for enrichment of training in ophthalmic pathology for ophthalmology residents at the Washington Hospital Center. These grants were still in effect at the end of the year and in addition, one grant for training in several basic sciences related to vision was activated at Columbia University.

One of the major developments of the year, was the establishment of the first NINDB Teacher-Investigator Development Grant at the University of Colorado Medical Center. This award for a Teacher-Investigator in Ophthalmology was made on behalf of Dr. Phillip P. Ellis.

2. Contributions Resulting from Particular Awards

As pointed out in last year's report, contributions resulting from support of research training, in terms of outstanding research performed by trainees, may not be discernible for a number of years after initiation of a training program. One immediate major beneficial result, however, has been the broadening of basic science training in clinical ophthalmology. Thus a number of clinical training

programs have added strong research and training groups in physiological optics, visual biochemistry, visual microbiology, and other basic science fields. This leads not only to immediately increased research in these areas by members of the training staffs, but augurs well for the training of well-rounded clinical researchers in the future.

3. Major Problems Encountered

The major problem in clinical ophthalmology continues to be the keeping of fellows, completing their training, in academic medicine and research. With reasonably adequate support available for living expenses through stipend support, however, the tremendous economic pressure resulting from debts accumulated during the postgraduate training period is somewhat alleviated. It is anticipated, therefore, that there will be less urgency for individuals completing training in clinical ophthalmology under NINDB training programs to go into the private practice of their specialty. Moreover, with the increased opportunity for academic careers becoming available through support of teaching and research positions with Teacher-Investigator Development Grants, it is anticipated that the fraction of fellows completing training in clinical ophthalmology who will remain in an academic and research environment will certainly increase.

Another major concern is: What constitutes good research training, and is there any "best" procedure for attaining this objective? A number of programs have increased the training period to four years, with one year, often the first, devoted largely, or sometimes entirely, to laboratory research pursuits. Whether this is the answer to good research training remains to be answered. The Vision Research Training Committee has taken cognizance of this problem and proposes to discuss it thoroughly.

4. Changes and Improvements in Program

A. The name of the Ophthalmology Postgraduate Training Committee was changed to the Vision Research Training Committee, to emphasize the broadened area of concern and major purpose of training.

B. The following members of the Vision Research Training Committee completed their terms with the September meeting:

Dr. Arthur Gerard DeVoe
Professor and Chairman
Department of Ophthalmology
College of Physicians and Surgeons
Columbia University
New York, New York

Dr. Edwin B. Dunphy
Chief of Ophthalmology
Massachusetts Eye & Ear Infirmary
Boston, Massachusetts

Dr. Michael J. Hogan
Chairman, Department of Ophthalmology
University of California School of Medicine
San Francisco, California

In addition, Dr. William F. Hughes, Chairman, Department of Ophthalmology, Presbyterian-St. Lukes Hospital, Chicago, whose term was due to terminate in 1961, resigned effective October 1, 1960.

C. Dr. Bernard Becker, Professor of Ophthalmology, Washington University, St. Louis, whose term of office as Chairman of the Committee was due to terminate in September, accepted reappointment as Chairman for an additional two-year period.

D. Dr. George Smelser, Professor of Anatomy, Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, accepted appointment to the Vision Research Training Committee for four years effective October 1, 1960.

5. Program Objectives for 1961

Program objectives for 1961 remain largely identical with those proposed for 1960. These are:

A. To bring about a better balance between basic science and clinical training programs. A considerable portion of programming activity during 1960 was spent in visiting basic science departments capable of providing good postdoctoral research training in basic science aspects of vision to acquaint them with the availability and purpose of NINDB research training grants. This activity has resulted in the submission of several training grant applications. It is proposed to continue such programming during 1961.

B. To place further emphasis on investigative pursuits in clinical training programs. Definite progress has been made in this direction in 1960. This problem will be further discussed by the Vision Research Training Committee. In this connection it is proposed to hold a conference of Program Directors, possibly during 1961 to review progress being made in research training under programs currently in effect, and to explore new approaches to attainment of training objectives.

C. To continue evaluation of program needs. One of the major innovations in 1960 was the initiation of the Teacher-Investigator Development Grant Program. Increased emphasis will be placed in 1961 on further development of this aspect of teaching and research.

6. Staff Assignment

Bernard Shacter, Ph.D.
Executive Secretary
Vision Research Training Committee

Elizabeth C. Hartman
Acting Head, Training Section
Extramural Programs

TABLE 1

SUMMARY OF COUNCIL ACTIONS

Clinical Ophthalmology Applications

Calendar Year 1960

MARCH	No.	Requested Amount	No.	Approved Amount
New or Revised	2	$ 58,725	1	$ 18,756
Continuations	3	178,897	3	176,953
Supplements	3	61,099	2	48,170
Total	8	$298,721	6	$ 243,888
Percent Approved			75%	82%
JUNE				
New or Revised	3	$106,398	1	$ 35,000
Continuations	3	120,909	2	60,621
Supplements	1	8,256	1	8,256
Total	7	$235,563	5	$ 103,877
Percent Approved			71%	44%
NOVEMBER				
New or Revised	1	$ 30,581	0	---
Continuations	2	139,505	2	127,625
Supplements	1	14,250	1	14,250
Total	4	$184,336	3	$ 141,875
Percent Approved			75%	77%
GRAND TOTAL	19	$718,620	14	$ 489,640
Percent Approved			74%	68%

SUMMARY OF COUNCIL ACTIONS

OTOLARYNGOLOGY APPLICATIONS

Calendar Year 1960

	Amount Requested	Amount Approved	Number Requested	Number Approved
MARCH				
New or Revised	$ 77,459	$ 43,972	3	2
Continuations	129,090	50,544	4	2
Supplements	32,875	26,306	3	2
Total	$239,424	$120,822	10	6
JUNE				
New or Revised	$ 59,657	$ 44,658	2	2
Continuations	237,225	111,545	6	4
Supplements	9,144	9,144	1	1
Total	$306,026	$165,347	9	7
NOVEMBER				
New or Revised	$ 49,494	$ 41,152	2	2
Continuations	109,424	43,525	3	2
Supplements	5,808	5,808	2	2
Total	$164,726	$ 90,485	7	6
GRAND TOTAL	$710,176	$376,654	26	19

III. SPECIAL FELLOWSHIPS (Traineeships)

1. Program accomplishments

This program of individual awards for advanced research training has continued to grow in 1960, as substantial numbers of trainees from Institute-supported Postgraduate Training Grant programs are motivated to enter the challenging field of research, with accompanying responsibility for training others, and to embark upon the additional years of specialized study necessary to produce a competent investigator.

A total of $1,915,560 was awarded to 213 trainees, an increase of approximately 30% over 1959. The international character of the training being provided by the program is attested to by the fact that awards have been made for study in 23 foreign institutions in Austria, Canada, Denmark, England, France, Germany, Italy, Japan and Sweden, and 55 in American institutions. A total of 151 outstanding laboratory and clinical scientists are providing guidance for these Fellows.

Previous postdoctoral training and experience of the 120 Special Fellows receiving initial support in 1960 ranged from 3 to 20 years, and averaged 7 years. Seasoned investigators continued to avail themselves of the opportunity presented by this program to develop investigative interests and sharpen or increase their research techniques; about 14% of the new awards this year were made to those with more than 10 years of postdoctoral experience.

Neuropathology continues to be an area in which this program is contributing to sorely needed manpower; 31 awards were made this year for training in neuropathology, an increase of 30% over 1959.

2. Contributions resulting from particular awards

Results of this program to provide special research training for future academicians are beginning to be evident in the appointment of former fellows to full-time academic positions in the award of Public Health Service research grants to former Fellows, and the frequency with which they are sought for vacancies which occur in medical faculties. The ever-recurring comment is heard that without National Institute of Neurological Diseases and Blindness Special Fellowship support, these individuals never would have considered it remotely possible to embark on the costly training required to fit them for positions in neurological research.

3. Major problems encountered

The outstanding problem encountered this year resulted from the merging of all fellowship and traineeship programs as fellowships. This is reflected in the change of program name to Special Fellowship instead of Special Traineeship. The accompanying procedural changes, which have been proposed but are not yet fully operative has given rise to some apprehension about the possibility of decrease in the usefulness of what is a most dynamic National Institute of Neurological Diseases and Blindness training program.

4. Changes and Improvements in program

The National Institutes of Health-wide consolidation of all fellowship and traineeship programs is the most radical change which has taken place. Whether or not this will result in improvement of the National Institute of Neurological Diseases and Blindness Special Traineeship program remains to be seen, once the new plan is in operation.

5. Program objectives for 1961

Expansion of the program will be continued, as availability of funds allows, and as is necessary to fulfil the demand for investigators in the neurological and sensory fields.

6. Volume of applications

Applications reviewed	336	$3,211,200
Applications approved	213	1,915,560
Average stipend	$8,700 per annum	

7. Staff assignments

Elizabeth C. Hartman
Acting Head, Training Section
Extramural Programs

Frederick Gutter
Training Analyst in charge of
Special and Postdoctoral Fellowship Programs
(since August 1, 1960)

IV. RESEARCH FELLOWSHIPS

1. Program accomplishments

This program of awards, designed to support research training of candidates qualified for investigative careers, but at less advanced levels than the Special Fellowship program continued at approximately the same financial level in Fiscal Year 1960 as in 1959, but with a redistribution of funds.

During the calendar year 1960, approximately $44,000 was awarded on an institutional basis to support 72 part-time students. Predoctoral Research Fellowships absorbed $72,947 for 21 awards. In addition $20,966 was expended for the support of 3 International Fellowships. The Institute took no part in the administration of either part-time, predoctoral or international fellowships beyond providing the funds for their support.

2. Contributions resulting from particular awards

Detailed information about the activities and contributions of research fellows subsequent to completion of support is unavailable to us.

3. Major problems encountered

All the problems encountered this year in administering the Postdoctoral Research Fellowship program in which we participate have arisen from frequent changes in National Institutes of Health policies and procedures for review and award of fellowships.

A number of the difficulties have been ironed out as they arose. However, the whole process for fellowship handling is very cumbersome in operation.

4. Changes and Improvements in program

As of July 1, 1959 the various Institutes were relieved of all responsibility for fellowships below the postdoctoral level. This has resulted in an augmenting of the funds available for Postdoctoral Fellowships to $580,000 for Fiscal Year 1961 from approximately $338,000 for Fiscal Year 1960.

5. Program objectives for 1960

It is anticipated that in the coming year procedures relating to Postdoctoral Fellowships may be stabilized, so that these awards may be utilized more effectively as an integral part of the Institute's total training program.

Program expansion is not contemplated at the present time, since substantial amounts are available from National Institute of Neurological Diseases and Blindness for research training at this level as stipends from Training Grant funds.

6. Volume of applications

Applications reviewed	100	
Applications approved	84	$530,124
Average stipend	$6,500 per annum	

7. Staff assignments

Elizabeth C. Hartman
Acting Head, Training Section
Extramural Programs

Frederick Gutter
Training Analyst in charge of
Special and Postdoctoral Fellowship Programs
(since August 1, 1960)

V. NEW PROGRAMS

As of July 1, 1960, two new programs were initiated, Senior Fellowship and Career Professorship Grants. An allotment of $100,000 for Senior Fellowships and $300,000 for Career Research Professorships was made from Fellowship funds.

Both types of grants will be made to an institution in behalf of an individual, to fill staff positions at two levels of responsibility. Senior Fellowship grants are to be made for 5 years, renewable for 3 years in addition, and Career Research Professorships for 5 years, renewable indefinitely.

Since information was not released until October, few applications have been received to the present time, and none have been reviewed or awarded.

Staff assignment

Elizabeth C. Hartman
Acting Head, Training Section
Extramural Programs

ANNUAL REPORT
Calendar Year, 1960
Perinatal Research Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

This report is presented in three parts:

1. A broad general statement briefly citing the major concerns of the Perinatal Research Branch relating to the refinement of the Study design, specific objectives, and the administration of the Collaborative Project.

2. A detailed activity report of the various sections of the Perinatal Research Branch.

3. Appendix containing tabulations prepared during the past year for use of the Perinatal Research Branch and for presentation to Project Directors and collaborators from the various contributing local projects.

I. GENERAL STATEMENT

During 1960, the Perinatal Research Branch has continued its function as the Central Office to the Collaborative Project on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood.

Experience with this Project prior to 1960 has revealed many problems which appear to be related to the collaborative nature of this endeavor, to the lack of more specific objectives or hypotheses to serve as adequate guidelines in the design, to the prospective approach to data collection which, despite many advantages over the retrospective approach, has its own difficulties, particularly in certain areas of this Study, and to the rather complex structure which relate the various collaborating units to each other and to the Perinatal Research Branch. Most of the activities of the branch have focused on these problems over the past year in an attempt to clarify these and assure maximal productivity of this Study.

Because of the multi-institutional nature of this Study, methods of data collection had to be carefully reviewed and evaluated to assure uniformity of procedure that could be met by the various collaborating institutions. The detail of this activity is spelled out at length in Part II of this report.

It has become increa

It has become increasingly evident that without rather specific objectives and guidelines, the central office is faced with a task which it cannot realize in view of its budgetary and personnel limitations. It is also questionable whether the participating collaborators could continue to handle the tremendous workload involved in the production of the desired research data of adequate quality for the remaining years of this investigation, without greater specificity of objectives.

It is evident that the data collected in this Project can be utilized for investigations which reach in part beyond the original broad objectives of this Study. The following outline may serve to clarify this statement.

1. The production of survey type data reflecting obstetrical practice and experience in fifteen collaborating institutions, obstetrical complications in 50,000 pregnancies, and detailed features of the physiology and pathology of reproduction in clinical terms based on direct observation of 50,000 pregnancies to name only a few possibilities. The research data already obtained and to be obtained in the seven year followup of Study children do reflect fairly detailed aspects of growth and development in such areas as physical and intellectual development, speech and language performance, but also the rather complete information about the general health of eventually as many as 40,000 children over the first seven years of life.

2. Specific studies of such questions as the prognostic implications of certain physical findings in the newborn for future development, the relationship of findings obtained by psychological evaluation of 8-months old children to intellectual development assessed at seven years of age, to give only two examples.

3. Retrospective investigations, once abnormal neurological outcomes of pregnancy such as cerebral palsy and the like are identified in Study children. The detailed prenatal records of the mothers of such children can be reviewed retrospectively to obtain clues and suggestions for the presence of patterns of problems related to specific abnormal outcomes. Such very meticulous clinical case studies based on rather detailed accurate information should yield valuable suggestions for further, more specific investigations.

4. To answer specific questions or test-given hypotheses about many factors operating during pregnancy which are suspected of being associated with cerebral palsy, mental retardation, and other neurological and sensory anomalies in the offspring.

In order to define the objectives of this investigation which can be realized without the danger of overburdening participating institutions beyond their capacity, and at the same time to identify very specifically such questions or hypotheses which this Study should address itself to, the Perinatal Research Branch has taken the initiative to clarify these

specific goals of the Collaborative Project, and is pursuing this task with the assistance of collaborators and consultants interested and knowledgeable in the field of perinatal research.

Specific hypotheses will address themselves to the following categories of pregnancy outcome:

1. Fetal deaths.

2. Neonatal deaths.

3. Infant and child deaths.

Obviously, these categories can be easily identified as events. However, because of the high autopsy rate of stillbirths and neonatal deaths which, in this Study, has been at least 90%, such outcome groups can be classified in most instances on the basis of detailed neuropathological findings and the detailed description of congenital malformations.

The classification of liveborns will utilize the following categories:

1. Prematurity by weight as well as gestational age. (This outcome category is particularly important for detailed investigation because of the role of prematurity as a factor in neonatal deaths as well as in brain damage in childhood.)

2. Congenital malformations of the central nervous system and other organ systems.

3. Manifestations of brain damage.

Category 3 will include:

1. Severe motor deficits (usually called cerebral palsy) which include spastic hemiparesis, spastic diplegia, athetosis, dystonia, and other related disorders.

2. Mental deficiency with or without neurological manifestations.

3. Sensory deficits, primarily of visual and hearing functions such as blindness and deafness.

4. Epilepsy or convulsive disorders with or without other neurological manifestations.

5. Speech and language disorders.

6. Learning and reading deficits.

7. So-called organic behavioral syndromes.

Categories 6 and 7 are only included at this time for the sake of completeness and certainly require further consideration in the light of adequacy of present methods of identification of such disorders in children up to seven years of age.

Specific objectives in the study of factors operating during pregnancy which are to be correlated with these outcome categories will focus on those factors which are known or suspected of being associated with abnormal pregnancy outcome on the basis of previous epidemiological investigations, primarily of a retrospective nature.

In addition to confirming or reevaluating such data from retrospective studies in a prospective investigation, it will be possible for many of these associations to elucidate the role of many subvariables such as specific obstetrical complications during labor and delivery, specific medical conditions in the gravida during pregnancy, specific medications given during pregnancy, virus disease and x-ray exposure during pregnancy, and so on, in order to pinpoint further the possible patho-physiological mechanisms which may be operative.

This will be possible because of the rather detailed and complete reporting of circumstances around each Study pregnancy, including the progression of pregnancy, the existence and nature of obstetrical and non-obstetrical complications, and the detailed progress of labor and delivery, as well as the behavior of the neonate in the early hours of life.

Specific objectives will also attempt to relate severity of specific illness rather than just the presence of such illness to pregnancy outcome. Since efforts are now being made to obtain complete information on medications taken during pregnancy, for non-obstetrical as well as obstetrical complications of pregnancy, it should be possible to decide whether a given disease or its treatment is associated with a given abnormal pregnancy outcome.

Although this statement about specific objectives of this investigation is incomplete, it nevertheless points out the many possibilities of this program which can be realized at this time. It is expected that these efforts will continue into 1961. Formulation of these specific objectives should provide the necessary stable framework for this Collaborative Project within which productivity can be most efficiently realized and predicted.

Brief reference should be made here to problems in data collection which relate to the prospective nature of this investigation. The production of Study data of adequate quality relating to events which have occurred prior to the enlistment of the woman in the Study is faced with problems. Such events include the past medical history, the past pregnancy history, and the family medical history. For all of these, verification of the reported information is desirable. For the family medical history, there is the additional complication that the woman is not reporting about herself but about relatives, and the report is thus second-hand. If one is willing to trust the reported information, the prospective approach will at

least avoid the bias in reporting which is expected to occur after the outcome of pregnancy is known. For certain variables with marked effect on pregnancy outcome, the reported data by themselves may be acceptable for analysis. In most cases, however, verification will be desirable. In order to reduce the volume of work, this verification may have to wait until a series of affected children have been identified. This procedure might be described as retrospective verification within a prospective study. The only problem would seem to be the loss of records in the intervening time and the death of relatives who might have been examined or who might have served as informants. It appears that the utilization of both the retrospective and prospective methods of approach would solve most of these problems, avoiding at the same time the burden of verification in a prospective manner.

During the past year the reorganization of administrative relationships within the NINDB resulted in the assignment of responsibilities for the Collaborative Project to the Perinatal Research Branch. The present organization of this branch is outlined in the attached chart.

In this organization, over-all planning and coordination is the function of the branch chief and his immediate staff. Two assistant branch chiefs have been assigned, one to coordinate primarily the intensive phase of the Collaborative Project, and one for the coordination of the extensive phase of this Project. Operations within the intensive phase are handled primarily by the newly organized sections: obstetrics, pediatrics, virology, laboratory studies and pathology, behavioral sciences, epidemiology and genetics, and statistical procedures and analyses. The position of a senior statistical advisor reporting to the branch chief has been planned and efforts are currently being made for the recruitment of an effective individual to fill this post. The Project Services Section includes staff members concerned with sample maintenance within the intensive phase, general medical literature services, and information activities.

During the past year, there has been an increasingly close working relationship between the Perinatal Research Branch and the Office of the Executive Secretary of the Perinatal Research Committee. The result is a marked improvement in relating to the total Study the mechanism for its financial support. With these improved communications, it seems possible to keep the reviewing committee more informed about the demands of the Study, and provide the collaborating institutions the necessary advice and guidance in mechanisms of requesting support.

Throughout the year, it has become increasingly apparent that this type of study requires an administrative support which is not easily compatible with existing administrative and organizational patterns and procedures of the NIH. The hybrid study, (i.e., one which of necessity must use both intramural and extramural methods and procedures) is beset with many administrative problems within the present NIH organizational structure. It has become quite apparent that many of the problems with which this branch has been faced and which reflect upon the efficiency of this

Collaborative Project are at least in part related to the use of administrative patterns which may not fit the specific needs of this program.

Efforts have begun during 1960 to define and prepare in written text a statement of the functions of the various components of the Study organization, i.e., the Perinatal Research Branch, the Perinatal Research Committee, the Project Directors, and other groups. Once this review has been prepared, it is hoped that this may form the basis for a reappraisal of administrative functions of the Perinatal Research Branch.

II. ACTIVITIES OF SPECIAL SECTIONS, PRB

I. Obstetrics Section

The primary activities of the staff concerned with the obstetric portion of the intensive phase have included the following:

A. Protocol improvements based on refinement of Study design, appropriateness of Study records for hospital use, and improved methods of securing and maintaining high quality of data.

B. Orientation and training of Project personnel.

C. Methods of handling forms and data.

D. Data production and analyses.

E. Evaluation of hospital performance.

F. Special Studies.

Specifically these activities may be outlined as follows:

A. Protocol improvement:

1. Revision of manuals.

2. Assistance to the Obstetrical Subcommittee in preparing revisions of manuals for the currently used Forms OB-30 through 33.

3. Revision of forms.

   The following forms have been revised and are now ready for pretesting: OB-12, 30, 31, 32, 33, 34 10, and a new laboratory form. The accompanying manuals have been prepared for each form.

4. An Obstetrical Coding Manual has been prepared for use in the Study in cooperation with Dr. James Donnelly and other obstetric consultants. In addition, the accompanying manual has been reviewed with Dr. Schuyler Kohl at a meeting in New York City on November 29, 1960.

B. Orientation and training of Project personnel:

1. During the year individual conferences were held with all Obstetric Coordinators. The quality of data and methods for improvement were discussed at these meetings. On June 3, 1960, the full Obstetric Committee met for a thorough discussion of the present status of this phase of the Project.

2. A two-day orientation meeting was held for hospital personnel concerned with the editing of obstetrical records on February 18 and 19, 1960. All hospitals but one were represented by the 30 participants.

3. To train all types of hospital personnel in the execution of the protocol, formal one-day classes were planned. To date, there have been a total of 13 of these classes, attended by over 100 physicians, nurses, lay observers, and editors.

4. In addition to the above activities, the majority of the hospitals were visited one or more times during the year by members of the central office staff engaged in the obstetrical phase.

C. Methods of handling forms and data:

1. During the year procedures for handling records and plans for the production of data were completely revised. Simpler and more economic methods for updating files were developed. At the time of this report, these changes have not yet been completed. All records on 12,000 cases have been refiled.

2. Earlier in the year special codes, together with editing and punching procedures, were devised for the following forms: OB-7, 9, 10, 31, 32, and 34.

D. Data Production:

1. Tabulations were produced from 7 forms which were analyzed and presented at the Project Directors' Meeting on September 26 and 27, 1960. Additional tabulations were reported on at the meeting of the Perinatal Research Committee on December 3, 1960 (see Appendix).

2. Processing of data from the gross examination of the placenta was initiated (see Appendix).

3. Numerous tabulations of selected cases were done in conjunction with the development of forms and codes.

E. Evaluation of records:

1. A protocol was developed for central office evaluation of records using medical personnel.

2. Procedures and a code are being developed for a clerical evaluation of the record to compliment the medical evaluation.

3. Hospitals have been evaluated on request and whenever special need has arisen.

F. Special Studies:

A study, still in progress, was initiated at the University of Tennessee to measure the validity of findings by physical examinations of obstetrical patients.

II. Pediatrics and Neurology

From January 1, 1960 to October 20, 1960 the Pediatric-Neurology Section has been engaged in the following activities:

A. Revision of Study data collection procedures.

B. Designing and analyzing special tests of reliability of Study data collection procedures.

C. Reviewing incoming Study records for quality control purposes.

D. Conducting training sessions.

E. Development of data handling and utilization methods.

All of these activities represent the combined efforts of the statisticians and physicians in the Pediatric-Neurology Section, and their consultants including the Pediatrics Subcommittee.

These specific accomplishments are as follows:

A. Revision of Study data collection procedures:

1. PED-1, Delivery Room Observations of the Neonate, revision dated October 1960 with manual.

2. PED-2, Neonatal Examination, revision dated May 1960 with manual.

3. PED-3, Nursery History, revision May 1960 with manual.

4. PED-5, Report of Tests and Procedures, new form dated May 1960 with manual.

5. PED-7, Summary of Hospital Course of the Neonate, revision dated May 1960 with manual.

6. PED-20, Follow-up Interval History, revision dated April 1960 with manual.

7. PED-10, Four-Month Pediatric Examination, revision dated October 1960 with manual.

8. PED-29, Summary of Medical Records, proposed August 1960 and not yet accepted for use.

9. CP-10, Report of Death (revision of PED-4), proposed August 1960 and not yet ready for use.

10. PED-12, optional Two-Year Pediatric Examination in preparation.

B. Special tests of reliability of data collection procedures:

1. Test-Retest study of PED-6, Neonatal Neurological Examination, conducted by three institutions, January through July 1960. Analysis in progress, certain conclusions derived but analysis not complete to date.

2. Test-Retest study of PED-11, One-Year Neurological Examination, conducted by three institutions, January 1960 to September 1960. Analysis in progress, certain conclusions derived but analysis not complete to date.

3. Test-Retest study of PED-1, Delivery Room Observations of the Neonate by two institutions, January to July 1960. Partial analysis performed and certain conclusions derived. Data not amenable to analysis as originally proposed.

4. Test of PED-20, Follow-up Interval History, April 1 through May 18, 1960. Analysis completed June 1, 1960, and report to Project Directors, June 17, 1960.

5. Analysis of correlations between examination findings on the Neonatal-Neurological Examination PED-6, diagnoses on PED-6, PED-2, and PED-7 and neurological status at Four-Month Pediatric Examination. Study, based on 2,500 cases, conducted June 15 through September 15, 1960. Analysis in progress but not completed to date.

C. Review of incoming Study records for quality control:

1. Samples of pediatric records from all institutions reviewed in April 1960 for report to the project site visit teams.

2. Review of samples of pediatric records from five institutions for Perinatal Research Committee use, July 1960.

3. Review of Follow-up Interval History records from all institutions, May 1960.

4. Review of Nursery Pediatric Records completed since July 1, 1960 from all institutions. Review done September-October 1960 for use by Pediatric Subcommittee and feedback to all instituions.

5. Specific communications to two projects and site visit to a third arising from the review mentioned in D.

D. Training sessions for pediatric lay editors:

Two 1-day training sessions conducted in April and May 1960 including editors from 12 institutions.

E. Development of data handling and utilization methods:

1. Development and use of two-card combination coarse code and item analysis code for nursery pediatric records January-October 1960.

2. Development of one-card index code for nursery pediatric records based on experience with previous code, presently recognized study needs, and present status hypotheses and data processing techniques.

3. Work in progress on utility of item analysis for diagnostic purposes, based in part on the experience with the study indicated in B-5 above.

4. Plans for one-card index code for all data between nursery discharge and one year of age, based on presently recognized study needs and present status of stated hypotheses and data processing techniques.

III. Genetics

In July 1960, Dr. V. Elving Anderson was appointed to the staff as Head of the Epidemiology and Genetics Section. His activities have included an evaluation of incoming genetics data, a definition of the most effective role of genetics within the Study, and the preparation of implementing proposals for revised methods and instruments. Dr. Anderson has met individually with numerous genetic consultants, many of whom had been involved in the early planning of this portion of the Study. He also evaluated certain series of cases from among those submitted by collaborating institutions, and abstracted the pertinent genetic data. A meeting of geneticists and some outside consultants was held at the PRB offices on October 27 and 28, 1960 to assist in the achievement of the tasks outlined above.

A meeting of outside consultants was held on November 14, 1960 to discuss the importance of blood groups in perinatal wastage. While implications for the Collaborative Study were of primary interest, the problem was considered in a broad sense so that it might be applied to other populations which may be available.

The major questions discussed were: (1) Maternal-fetal incompatibility with respect to the ABO system, and (2) Heterozygote advantage (and homozygote disadvantage) with respect to the ABO and the MN systems. For satisfactory analysis, data must be available on mother, father, and child (and data on all living siblings of this child would be very desirable). Tests for salivary secretion of ABH and Lewis substances should also be done if possible. It was thought that a sample of 5000 families would be adequate for analysis.

A study of this type has already been initiated by Dr. Fred Allen of Boston using families enlisted in the Collaborative Study there. By the time of the November meeting over 500 fathers had already been tested. The group felt that this was a very suitable population and that suitable steps should be taken to help Dr. Allen secure the research funds necessary for him to continue and expand his program.

The group was very enthusiastic about this type of study and felt it would make a significant contribution to our knowledge about blood groups, population genetics, and perinatal wastage.

Dr. Anderson and his staff, following these exploratory discussions, are developing proposals for the revision of the approach to genetic information obtained within the intensive phase of the Study, and proposals for supplementary genetics studies.

IV. Socio-Economic Information

A meeting of representatives of the collaborating institutions was held in PRB offices in January 1960 to consider the socio-economic information required to meet the objectives of the Collaborative Project. Participating personnel included social workers, interviewers, editors, field directors, etc. Discussions at this meeting were concerned with an operational evaluation of the forms and instructions then in use and a discussion of methods for their refinement. Shortly thereafter, a subgroup for outside consultants was appointed to assist in the re-evaluation of this protocol. The subgroup included: Dr. Sidney Goldstein, Professor of Sociology, Brown University, Providence, Rhode Island; Mr. Joseph Dallas, Field Director, Johns Hopkins Hospital, Baltimore, Maryland; Dr. Katherine French, Anthropoligist, University of Oregon Medical School, Portland, Oregon; Mrs. Laura Sharpe, Bureau of Social Science Research, Washington, D.C.; Dr. Howard Kellman, Research Associate, New York Medical College, Metropolitan Hospital, New York; Miss Katherine Murphy, University of Minnesota Medical School, Minneapolis, Minnesota, and appropriate PRB staff members. The subgroup, meeting for two days in May 1960 and again in August 1960, reviewed tabulations of data received within the Study, reviewed the primary objectives of these particular data, and made specific recommendations for the revision and clarification of the socio-economic protocol. PRB staff members implemented the recommendations of the subgroup, prepared the appropriate revised form and manual of instructions, and in December of 1960 distributed these proposals for pretest by all collaborating institutions.

V. Evaluation of the Attitudes of the Pregnant Woman

A staff member in the Behavioral Sciences Section has been assigned to the area of the evaluation of the attitudes of the pregnant woman. She has visited and maintained continuing contact with staff members in collaborating institutions who have been interested in this area, many of whom have been conducting studies employing a variety of techniques. In June 1960 a meeting was held in PRB offices of some of these staff members, together with outside consultants, to discuss results available at that time. Participants reported on their own experinces with a variety of techniques and discussed the possible application of such techniques to the Project. Recognizing that heavy logistical demands of some methodologies would not allow for their use within the Collaborative Project, there was discussion of development of a simple self-administered questionnaire for Project purposes. Dr. Earl Schaefer, one of the meeting participants, discussed his own experience with such a questionnaire and indicated his willingness to work with PRB staff members in the further development of something for Study purposes. Following this meeting, a questionnaire

was drafted which incorporated some items from Dr. Schaefer's scale, from other investigators' reports, and some originated by PRB staff members. The questionnaire consists primarily of items dealing with psychosomatic reactions, fears for the self, desire for pregnancy, fears for the baby, dependency, mood, and some culturally-oriented questions. Recent project site visits by PRB staff have been concerned with putting this scale, currently in a "comment draft form", into a pretest phase at Minnesota and Oregon. In addition, Boston Lying-in Hospital, Charity Hospital and the University of Virginia Hospital have all agreed to gather some data with this instrument. It is to be hoped that Memphis and Philadelphia will join in this pretesting soon. In the near future both Columbia and Metropolitan Hospitals in New York will also be asked to participate in this pretesting period.

VI. Past Medical History

A. The procedure for conducting the Past Medical History (Forms OB-2 through 8) to date is still that which was introduced in February 1959. In an effort to provide systematization and standardization of questions directed to gravidae, members of the PRB and Johns Hopkins Hospital cooperated in the development of draft manuals during the period March-May 1960.

B. A pretest of these draft manuals, with forms unchanged, was conducted between July 18 and November 21, 1960. Although the manuals were considered helpful for training purposes, their use with the February 1959 record forms created other problems, i.e., coding, ease of administration, etc.

C. Although the use of the structured interview technique is indicated, modification of the current items on OB-2 through 8 forms is required. On the basis of a series of meetings attended by Drs. Berendes, Leavitt, Vollman, Mendelson and Mr. Hoover and Mr. Morrone, elimination of some of the current obstetrical interview forms appears advisable. This decision was based on returns from these forms indicating that the data therein are open to some question as regards validity and reliability. Further presentation of some of the problems in this area was made in a joint meeting with the obstetric subcommittee in December 1960.

At the present time the approach to the revision of the Past Medical History protocol envisages a shorter interview, concentrating on major events in the gravida's life, e.g., operations, major illnesses, etc., for which hospitalization or visits to a physician were required. These items would be limited to a specific time period (2-3 years prior to

enlistment as a Study case) and every effort would be made to obtain verification of all such events.

VII. Viral Serological Studies

In July 1960, Dr. John L. Sever joined the PRB staff and was subsequently appointed Head of the Virology Section. During 1960 the staff of the Virology Section and Serum Center has been expanded to meet the increasing workload. The joint operation of these two units has been effectively organized and coordinated during this same period. Serum collection procedures involving the various collaborating institutions have been further clarified and standardized during recent months. The team of virology technicians, originally organized by Dr. R. Huebner of NIAID, was placed under the direction of the Head, Virology Section and has become an integral part of the PRB operation.

The instructions to collaborating institutions for collecting, processing, and shipping blood serum for this Project were revised and discussed with Project Directors at their meetings in June and September of 1960. Further minor revisions have been proposed and it is anticipated that the final version of standard instructions will be approved for use in January of 1961. Similarly, proposals for the revision of the schedule for drawing blood from Project patients was discussed with Project Directors in June and September of 1960, and it is anticipated that a more meaningful schedule, which is also generally acceptable, will be adopted by January of 1961.

Throughout 1960 PRB staff responsible for the operation of the Serum Center have reported on the extent to which collaborating institutions were meeting simple requirements of supplying Study blood samples, primarily in terms of adequate quantity and quality of serum (i.e., without hemolysis or cells). During the latter months of 1960, the technicians of the virology section have begun preliminary analyses of samples of Study blood to assure its adequacy for later analysis. Inadequacy of quantity or quality of blood serum has been reported routinely to collaborating institutions, so that necessary corrective measures may be taken when indicated.

VIII. Pathological and Laboratory Studies

A. Pathology

1. A protocol for the handling of fetal material equal to or more than 16.5 cm. crown-rump length was developed and approved.

2. A protocol for the handling of fetal material less than 16.5 cm. crown rump length was developed and approved.

3. The classification and a storage system at PRB for all pathology specimens contributed by the collaborating institutions was completed.

4. The recording of all deaths, and their classification by cause was accomplished.

5. A detailed investigation of the possible use of the examination of deciduous teeth in the Project was reported.

B. Neuropathology

1. A protocol for the handling of neuropathological material was developed and adopted.

2. The receipt and processing of over 120 brains was accomplished.

3. A program to study the external morphology of the cerebral cortex was developed.

4. Discussions were held with neuropathologists on histochemical techniques which may be applicable to certain specimens.

5. A neuropathology microscopy room was established in the Robin Building.

C. Special Studies

1. A protocol was developed for standardization of ABO, Rh, and Coombs tests.

2. A detailed investigation of the accuracy of bilirubin determination was completed and reported (see Appendix).

3. Seminars were held in the study of normal neuroanatomy and neuropathology.

IX. Interval History

The Collaborative Project study design includes periodic followup Interval History interviews to secure information about those events and situations in the child's life which may have caused brain damage or to discover symptoms or behavior patterns which may indicate brain damage.

A subgroup to consider this aspect of postnatal followup was created in late 1959. Its members included Dr. Zanwill Sperber, Psychologist, Philadelphia Children's Hospital; Dr. Robert Dorsey, Psychologist, Charity Hospital, New Orleans; Dr. James Drorbaugh, Pediatrician, Boston Lying-in Hospital; Dr. Betty Hemphill, Pediatrician, Johns Hopkins Hospital, Baltimore; Dr. Jerry Solomons, Pediatrician, Brown University, Providence, Rhode Island; Dr. Rosaline Goldman, Psychologist, NINDB; Dr. H. Douglas Jameson, Medical Officer, NINDB, and Dr. John A. Lynch, Medical Officer, NINDB.

During the first half of the year 1960, this group met bimonthly. Form PED-20 was put into pretest with each member of the committee visiting at least one COLR institution to supervise the administration of PED-20 and the effectiveness of the use of the interview guide developed by Dr. Sperber. The experiences of the group were pooled during a two-day meeting at Brown University and changes were decided upon and incorporated into the PED-20 which was designed for use at the 4, 8, and 12 month examinations.

Several hundred records were examined by the various committee members. The psychologists focused on the social-developmental items and the medical members of the committee focused on data dealing with reports of illness and considered the clinical value of these reports. The results of the analyses of the data were presented to the Project Directors at their meeting in June 1960. In the succeeding meetings of the Interval History Committee the items aimed at eliciting medical information were further clarified. A form for requesting medical information from physicians was then developed. The PED-20 form currently in use was adopted by the Project in June 1960. The committee has a directive from the Project Directors that they work on a PED-20 form for the 18 and 24 month-old Project children.

X. Psychological Examination of the Infant

A. The collection of standardization data for the Bayley tests of Mental and Motor Development was completed in April 1960. Eleven COLR institutions contributed a total of about 1200 records; central staff psychologists participated in the standardization testing from December-April 1960. Analysis of the standardization data was undertaken in May and June. New age-placements became available in July 1960. They provided a framework against which to evaluate the development of "normal," "suspect" and "abnormal" children in the COLR study.

B. Reliability studies were undertaken at NIH by central staff psychologists from May-July 1960, and by psychologists at six COLR institutions from August-September 1960. They included tester-observer and test-retest reliability studies. Analysis of the tester-observer data was completed in October. The reliability studies allowed the selection of items on the 8-month examination which could be administered and scored uniformly in different institutions. Those items which needed clarification in the directions for administration and scoring were identified and those requiring examiner's judgment (and were therefore too subjective) were eliminated.

C. Item-total score and inter-item correlations on the mental and motor scales were computed on 8-month records in November and December 1959. The inter-correlations yielded

clusters of items which will aid in the ordering of scale content. A profile can thus be provided which summarizes the successes and failures of infants in specific areas, such as vocalizations, imitations, memory for hidden objects, goal-directed motor-activity, etc.

D. Two analyses were done to find out whether the performances of "suspect" and "abnormal" babies differ significantly from that of matched controls on the Mental and Motor Scales and the Infant Behavior Profile. Dr. M. Honzik, working on an NINDB contract in California, reported in Chicago, September 1, 1960, on a comparison of "suspect" and "normal" babies. Similar analyses were undertaken for "abnormal" babies from Project units in Boston and Philadelphia in the central office in August and September 1960. Matching with "normal" babies was done by age, sex, parity and examiner. It was found that "abnormal" and "suspect" babies differ on total scores and specific items on the Mental Scale, Motor Scale and the Infant Behavior Profile.

E. Communication has been improved via three channels:

1. Five meetings of COLR psychologists (one of the total group, one of all Eastern COLR psychologists, three of the psychology subgroups described below).

2. Four workshops for training new psychologists (two at NIH, two at the Institute of Human Development, University of California).

3. Site visits undertaken by central staff psychologists to all but one COLR institution.

F. In April 1960 a Psychology Subgroup was established to participate with central office psychologists in planning and implementing the program of psychological examinations in the COLR study. Present members: Dr. Miriam Fiedler, Boston Children's Hospital; Dr. Raymond Holden, Brown University; Dr. Ethlyn Klatskin, Yale University; Dr. Harold Michal-Smith, New York Medical College; Dr. William Von Lackum, University of Tennessee.

In the past five months this group has completed a revision of the 8-month examination. General aims have been to relate the various parts of the 8-month examination more closely to the objectives of the core project, to make them more appropriate to the age under study and to make them less time consuming. Members of the subgroup developed instructions and manuals to increase uniformity in

administration and scoring of the 8-month examination, devised descriptions of behavior that would lead to objectivity in observation and evaluation, and designed forms that would simplify recording and coding. Specific changes made in the revision of the 8-month examination are listed below:

1. A procedural manual is provided which includes general instructions for the conduct of the entire 8-month examination.

2. Changes in the Scales of Mental and Motor Development have been aimed primarily at the elimination of redundant or ambiguous items, at clarifying directions for the administration and scoring of the remaining items and at their proper age-placement. New test forms have been prepared.

3. Only those dimensions were kept in the revised Infant Behavior Profile which seemed relevant to the diagnosis of brain-damage and appropriate to the age under study. A new form, Additional Observations, was developed for use with the Infant Behavior Profile to provide information on sensory-motor development not obtained with the developmental scales. Manuals for both forms have been prepared.

4. Rating scales were developed to encompass those facets of the mother's behavior which could be observed during the testing of her eight-month-old child. Her expressed attitudes and her behavioral reactions to and with the child as well as her responsiveness to the child's needs are to be rated. The manual gives examples of the situations in which this behavior may be observed.

G. A pretest of the revised 8-month examination was undertaken by eight COLR institutions in August and September 1960. At the last Psychology Subgroup meeting on October 20 and 21 the results of this pretest (including tester-observer reliabilities and institutional differences) were presented and discussed and the final form of the revision was drawn up. Its adoption by all COLR psychologists and the Project Directors in early 1961 is anticipated.

H. A review of the literature has been undertaken which has aided in the revision of the 8-month examination and in planning for future examinations. The literature has been

searched with regard to:

1. The emergence of "age-bound" behavior characteristics in young children from infancy through early school years.

2. Individual reaction patterns which are relatively age-free, but which might be relevant to the diagnosis of brain-damage and which promise some consistency in predictions made from early childhood to a later date.

3. Psychological tests specifically designed, or showing promise for differentiating children with CNS damage from normal children of preschool and early school age.

I. Planning for a psychological examination of the child at the age of 4 years has begun. PRB staff members, working with the psychology subgroup, plan to develop an instrument which will provide a global estimate of intelligence as well as specific tests designed to disclose brain damage. These staff members have been consulting with outside investigators such as Dr. E. Meyer Taylor, Dr. Francis Graham and others who have had special experience in efforts to detect brain damage in preschool children.

J. Contemplated in the near future are:

1. The coding of the 2000 eight-month records which have been received by now at the central office.

2. A comprehensive analysis of test records of all babies which have been diagnosed so far as "abnormal" or "suspect" during any of the examinations given during the first year (neonatal, pediatric, psychological and neurological). It is hoped that this group may be separated into subgroups with specific diagnostic syndromes and corresponding test behavior patterns.

3. Comparison of the data on social development of the child as reported by the mother in the 8-month interval history (PED-20) with the behavior of the child as rated by the psychologists during the 8-month examination.

XI. Speech and Hearing Evaluation

Consultants in the area of speech and hearing were invited to meet in PRB offices on May 25 and 26, 1960 to discuss the development of an examination of the Study child at some age between 1 and 6 or 7 years. It was recommended that such an instrument be developed for the age of 3 years

and recommendations were made by the consulting group as to the areas which should be included in this examination. Each of the consultants prepared portions of a draft examination, sections of which were pretested in September, October and November of 1960 at Johns Hopkins Hospital. The consulting group convened in PRB offices again in early December of 1960 to review the experience of the pretest, and make further plans for the development of this examination. One member of the psychology subgroup was selected to serve as a liaison between these two bodies of consultants, since there is some overlap in content and technique. Further revision of the original examination is now being accomplished by individual assignment among the outside consultants, and it is anticipated that this group will reconvene in February or March of 1961. The PRB staff expects that the draft of an examination document, report forms, and instructions will be available by late spring or early summer of 1961. This will allow for pretest during the summer and early fall of 1961 and final printing and distribution of forms and manuals to collaborating institutions before the end of 1961. Since the oldest Study babies will have reached the age of 3 in the early months of 1962, this schedule is mandatory.

XII. Sample Maintenance Activities

In April 1960 a statistician was employed whose past experience included field operations and the use of public relations techniques. His principal assignments were (1) to develop a uniform system of reporting sample maintenance rates from the collaborating institutions and (2) to provide assistance to collaborating institutions in sample maintenance techniques, develop channels of communication for the exchange of ideas and experiences among institutions and to develop other activities to promote the most effective maintenance of the Study sample.

Subsequently, information has been collected and disseminated concerning the organizational structure, operating procedures and incentives employed by the 15 collaborating institutions in sample maintenance programs. Proposed systems of reporting have been discussed with all collaborators and a uniform method of determining rates has been developed. Exchange of information among collaborating institutions has been achieved through frequent field trips by this staff member, through correspondence, and during a two-day meeting held on November 9-10, 1960 in the PRB offices with sample maintenance personnel from all institutions participating.

XIII. Information Activities

Information activities relevant to the Collaborative Project have been conducted by an Information Officer assigned to PRB from the Information Office of NINDB.

These have included:

A. Five issues (a total of 42 pp.) of the Collaborative Project Reporter were published and distributed to

all Study staff members.

B. The leaflet "You and Your Baby" was published and 50,000 copies were sent to the 15 Project Directors for distribution to Study mothers.

C. The leaflet "Careful, Accurate Interviewing" was published and 5,000 copies were sent to the 15 Project Directors for distribution to Study staff members.

D. The exhibit "The Fateful Months When Life Begins" was produced and shown 12 times. (It won an award at the 109th annual meeting of the American Medical Association.) In addition, the old Project exhibit was shown 5 times, and a new, small exhibit was produced for future use.

E. A progress report, entitled "Special Report: Perinatal Studies," was revised and copies are now being sent to all Study staff members.

F. Kits of information materials relating to the Project were sent to all Study personnel and to about 500 non-Study physicians and allied specialists throughout the world.

G. Arrangements were made with the Smith, Kline, and French Laboratories Film Center to distribute the film "Resuscitation of the Newborn" to the 15 Project Directors.

H. Prints of the films "Year of Birth" and "Neurological Examination of the Newborn" were sent to about a dozen individuals and organizations.

I. A speech on the Project was written for a government official.

J. Inquiries from six members of the press were received and answered.

K. An orientation manual, entitled "Introduction to the Collaborative Project," for Study staff members.

L. Two posters, one addressed to Study staff members, the other to Study mothers.

The following information materials are now in production and will be distributed, as indicated, during the next six months:

A. A special, illustrated edition of the Study manual "Examination of the Placenta," for non-Study physicians.

B. A leaflet, "When You and Your Baby Come Home," for Study mothers.

C. A leaflet, "Hospital Personnel and the Collaborative Project," for non-Study employees at the collaborating institutions.

D. A leaflet "The Physician and the Collaborative Project," for non-Study physicians.

E. A certificate of appreciation, signed by the Secretary of Health, Education, and Welfare, for Study mothers.

F. Press kits, for the 15 Project Directors.

G. A leaflet, "The Collaborative Project: A Nationwide Research Attack Against Brain Damage in Children," for the general public.

H. Bimonthly issues of the Reporter, for Study staff members.

XIV. Data Processing Procedures

Development of the methods to be used in operating a complete inventory of forms system and forms tickler system was completed during the year. Programming of the system to be done on a Minneapolis Honeywell - M800 computer is under way. Access to the computer is expected in May 1961 and delivery of NIH's own computer equipment is scheduled for autumn 1961. The system is expected to be in full operation in June-July 1962 based on the above dates.

As part of the reorganization of functions within the PRB, a change was instituted from centrally operated coding and filing procedures to individual section-controlled procedures. This change-over will be fully completed by the end of the first quarter of 1961.

New approaches were developed both for the processing of Study forms and the control of Study sampling procedures.

XV. Activities of Medical Literature Services

A. Prepared punch cards and reproduction multilith mats for the following reference lists on:

1. Radiation (44 pages) (1950-1958)

2. Placenta (18 pages) (1957-1960)

3. Emotional Factors in Pregnancy, etc. (5 pages) (1958-1959)

These were distributed in May. Written and phone requests have continued to date for these lists.

B. Correction and collation of the two papers by Professor Luria, as recommended by Dr. Myklebust. (Each paper was 28 pages long.) Approximately 125 copies were prepared and forwarded to Dr. Myklebust, and about 200 copies prepared for the Collaborative Research staff and NINDB.

C. Several conferences with Dr. Rogers (Director of the National Library of Medicine)and Dr. Brodman (Dr. Rogers' assistant) which resulted in the willingness of the NLM to produce for the Perinatal Research Branch, a five-year bibliography in topics of interest to the Branch, for a cost of $6,000 and to be completed by (or about) January 1, 1962.

D. Short bibliographies "on demand" individually requested by COLR staff members were also prepared in the areas of:

1. Seasonality and birth defects.

2. Bacteriuria in pregnancy.

3. Listeria in pregnancy and newborn.

4. Thyroid defects and mongolism combined.

5. Birth weights, duration of gestation, statistics on.

6. Meperidine and morphine, effects on the newborn.

7. A continuing collection of references on the subject of epidemiology of cerebral palsy, congenital malformations, CNS birth defects, etc., is being regularly compiled.

E. During the summer 1960, the addition of two clerk-typists and a COSTEP (medical student) (all for the summer only) enabled a speed-up in the MLS work program so that two new reference lists were begun and completed during this period:

1. "The Role of Vitamin C in the Etiology of Congenital Neurological Disorders and Perinatal Mortality." (1954-1960) (31 pages); and

2. "Infant Mortality and Morbidity". (1957-1960) (56 pages).

F. Work on new reference lists continues in the following areas:

1. Thyroid function in pregnancy and pregnancy outcome--an extensive listing of over 800 references of the relationship of thyroid function in pregnancy and perinatal disorders in the newborn.

2. Prematurity--literature currently being searched and punch cards prepared. (About 50% completed.)

3. Cerebral palsy, congenital malformations, and abortion, etc.-- literature currently being searched and punch cards prepared. (About 25% completed.)

G. For COLR staff use, a Kardex file of ongoing research has been set up covering over 750 research projects related to perinatal studies, and categorized in over 50 subject topics. This is a continuing project.

H. Progress is also being made in the development of a coding system of subjects or subject titles to be used in a mechanical information retrieval system and which will tie in with other universally used sources of medical and research information.

ANNUAL REPORT
Calendar Year 1960
Biometrics Branch - Collaborative and Field Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

A. SCOPE OF PROGRAM

The interests and involvement of the Biometrics Branch during the calendar year 1960 may roughly be divided into three major areas:

1. Collaborative Project of Cerebral Palsy and Other Neurological and Sensory Disorders of Infancy and Childhood.

2. Statistical consultation and/or service given to investigators outside of NINDB on other projects.

3. Statistical consultation and/or service furnished to clinical and basic research investigators at NINDB in the areas of neurology and blindness.

Accomplishments achieved and problems encountered during the course of 1960, as well as proposed future objectives in each of the above three areas, are reviewed below:

1. Collaborative Project of Cerebral Palsy and Other Neurological and Sensory Disorders of Infancy and Childhood.

In January 1959, "final" study forms covering the various aspects (prénatal, labor, delivery, neonatal, etc.) had been distributed to each of the participating institutions. Where an institution's case selection procedures, the quality of its data, collection procedures, and its completed forms were considered both by the institution and the central office staff to be of sufficiently high calibre, it was permitted to start enrolling so-called "final" study cases. By the end of 1959 all but three of the institutions had started to enroll such cases. As 1960 progressed, however, even these three institutions had improved sufficiently in the quality of their procedures and completed forms to enable the central office to permit them to enroll study cases. As of September 30, 1960, some 11,462 gravida and 6,227 neonates had been processed as "final" study cases.

During the year, members of the Biometrics Branch, assisting medical and other professional personnel in the Collaborative Project, accomplished the following:

a. Prenatal and Obstetrical Areas:

1. Protocol Improvements

(i) Six "old" forms and one new one were revised and prepared for pretest. The corresponding manuals of instruction were also revised.

(ii) Five forms are being revised or prepared for pretest.

2. Orientation and Training of Project Personnel

(i) During the year statisticians and consultants held individual conferences with all obstetric coordinators to discuss quality of data and methods of improvement of such data.

(ii) A two-day orientation meeting was held for hospital personnel concerned with the editing of obstetric records. All hospitals but one were represented by the 30 participants.

(iii) In order to train all types of personnel in completion of the study forms, eight formal one-day classes have been held which have been attended by 60 physicians, nurses, and medical editors.

3. Methods of Handling Forms and Data

(i) During the year procedures for handling records and plans for the production of data were completely revised and more economical methods for updating files were developed. These changes have not been completed to date.

(ii) Work was begun on new codes to supersede the present coding procedures.

(iii) Additional codes for six forms, together with editing and punching procedures, were devised for use with records as they were filed.

4. Data Production

(i) Tabulations were produced from seven forms. Brief analyses were undertaken and findings reported to the Project Directors.

(ii) Processing of data from the gross examination of the placenta was initiated.

(iii) Numerous tabulations of selected cases were done in conjunction with the development of forms and codes.

4. Evaluation of Records

(i) A protocol was developed for central office evaluation of records, using medical personnel.

(ii) Hospitals have been evaluated when the specific need has arisen.

(iii) Procedures and a code are being developed for a routine clerical evaluation of each record.

6. Special Studies

(i) A study, still continuing, was initiated at the University of Tennessee to etermine the validity of findings on physical examination.

b. Pediatric and Neurological Areas:

1. Protocol Improvements

(i) Seven forms were revised or prepared preliminary to pretest. The corresponding manuals of instruction were also revised.

(ii) Three forms are being revised or prepared preparatory to pretest.

2. Orientation and Training of Project Personnel

(i) Assisted in planning and conducting two workshops for pediatric lay editors.

3. Methods of Handling Forms and Data

(i) Developed pediatric codes.

(ii) Supervised and instructed central office pediatric statistical clerks in the processing, editing and coding of pediatric records. More than 5,000 sets of nursery records have been coded to date, with approximately 10,000 punch cards being produced.

4. Data Production

(i) Some 60 pages of detailed tabulations were produced from two forms on approximately 3,000 neonates.

These tables were distributed as "feedback" to the collaborating institutions.

5. Evaluation of Records

(i) Evaluations of pediatric forms were prepared for each collaborating institution.

6. Special Studies

(i) Test-retest studies were set up for the following examinations:

Revised Neonatal Neurological Examination, 2 institutions, approximately 350 cases

Revised Delivery Room Examination, 2 institutions, approximately 270 cases

One-year Neurological Examination, 3 institutions, approximately 100 cases.

(ii) Pilot study to compare nursery findings with neurological diagnoses at four-month examination, eight institutions, approximately 2,500 cases.

(iii) Study of Buffalo Child Growth Study data to determine age at which neurological abnormalities are first detected. This analysis was completed and the report presented to the Pediatric Subcommittee.

c. Genetic, Socio-Economic, and Psychology Areas:

1. Protocol Improvements

(i) Assisted in proposed revision of socio-economic data collection form and manual.

2. Orientation and Training of Project Personnel

(i) Trained personnel from collaborating institutions in abstracting and tabulating genetic data reported in family health history and obstetrical data sheets.

3. Methods of Handling Forms and Data

(i) Prepared editing and coding instructions for socio-economic data.

4. Data Production

(i) Prepared tabulations for presentations at two genetic meetings.

(ii) Prepared summary report of genetic findings for Project Directors.

(iii) Prepared tabulations for presentation at socio-economic data meeting.

(iv) Prepared special tabulations of socio-economic data reported on family health history data sheets.

(v) Prepared summary report of socio-economic findings for Project Directors.

(vi) Tabulated data recorded on 8-month psychological examination of child, including a report on editing problems, special comparisons of "damaged" and "normals," tester and institutional differences, etc.

(vii) Prepared special tabulations and reports on twinning and consanguinity.

5. Evaluation of Records

(i) Evaluations of genetic, socio-economic and psychological forms were prepared for each collaborating institution.

d. Sample Maintenance Area:

During the year activities relating to sample maintenance were started. This program had two basic objectives: (a) the establishment of procedures and incentives aimed at insuring the highest rate of retention of participants for the duration of the Collaborative Project, and (b) the provision of the uniform system of reporting by hospitals to the central office in matters pertaining to rates and other assessments of separations from the study. In order to make available to each institution the programs and practices established elsewhere and to minimize loss of patients from the study, a questionnaire requesting this information was forwarded to each institution. As a result of information derived from this questionnaire and from other sources, a proposed report form to be used by all institutions in reporting periodically the total number of study cases to date, the number and type of appointments, and, where appointments were not kept, the reason for such failure. This report form was presented to the Project Directors for approval with the understanding that each institution remained free to maintain as much additional information as desired.

A revision of the report form was accepted by the Project Directors, subject to further consideration in staff conferences at each hospital. The area of sample maintenance is one that is vitally important to the success of the study. It will need careful development and implementation at each institution.

The largest single effort of the Biometrics Branch relating to the Collaborative Project is the processing of data collected in the course of the study. In the development of mechanisms for handling and processing the great quantitites of data, it has, of course, been necessary to insure that new or revised forms, as well as procedures for editing and coding such data, be reviewed with respect to suitability for data processing.

The Biometrics Branch was requested, as a temporary measure, to establish necessary direction and control needed in the design and printing of forms used in the study.

Substantial progress has been made in the development of a comprehensive tickler system for use in connection with the Minneapolis-Honeywell 800 computer to be installed at NIH in 1961 to check on the receipt of specified study forms for each study gravida and child.

Planning is under way in connection with the development of programs to be used for the mechanical production on the IBM 650 of chi-square and multiple correlation statistics for use in the study.

Punch cards prepared to date from obstetrical administrative records for study and non-study cases have been useful in getting distribution of gravidas by age, race, and gestational age at time of registration. This allows a comparison between study and non-study gravidas to determine whether or not the gravida study sample is drawn without bias from the sampling frame.

In order to arrive at some crude measure of the expected repeat pregnancy experience in the collaborating institutions, three hospitals furnished the Biometrics Branch with punch cards of gravidas covering a pre-study period. Although it was recognized that analysis of such data could not be generalized to all institutions, and although the present hospital experience may differ from that of several years ago, these data have indicated that about 20% of the patients delivering in a given year could be expected to return to the hospital for at least one other pregnancy within the next five years. No allowance can, of course, be made for the effect of the Collaborative Project itself on the tendency of the gravida to return to the collaborating institution when she is again pregnant.

During the course of the year the Branch Chief attended some meetings of the Perinatal Research Committee. He and other Branch members attended meetings of the Project Directors, as well as conferences and workshops of obstetricians, pediatricians, neurologists, speech and hearing consultants, psychologists, geneticists, etc. In addition, numerous meetings of small

working groups and special meetings with outside consultants were attended by staff members in the capacity of statistical consultants.

A meeting of Project Statisticians and other personnel responsible for the biometric aspects of the Collaborative Project at the local level was held in the central office in May 1960. Data available from three study hospitals on past repeat pregnancy experience were reviewed. Various approaches to sampling of repeat pregnancies and the types of problems in each approach were discussed. Also distributed and reviewed were obstetrical administrative data from all hospitals reporting study cases during calendar year 1959, as well as comparisons of distributions by race, gravida's age and gestational age at time of registration, and marital status of study and non-study cases for six study hospitals, covering the last three months of calendar year 1959. The inadequacies of the present obstetrical administrative form in furnishing data to meet special group sampling needs were discussed. The group was brought up-to-date on the recommendation of the Perinatal Research Committee concerning "walk-ins."

Every institution in the Collaborative Project was visited at least once during the year by Branch staff. The purpose of these visits was to obtain information regarding the characteristics of the hospital population, case selection procedure, selection of children for standardization of the Bayley psychological test, processing of patients, completion and editing of study records, sampling maintenance procedures, and other problems of a statistical nature with regard to the various aspects of the Study as well as specific methodological and ancillary investigations. These visits tended not only to "educate" personnel in the collaborating institutions in the statistical aspects of the study, but also to enhance the appreciation of the Branch staff of the difficulties encountered in the application of some study forms and of the need of possibly different approaches.

Branch members have worked closely with medical and other professional members of the Perinatal Research Branch, NINDB, in setting up study forms suitable for processing and data reduction, and devising codes and manuals of procedure and instruction. The staff also prepared the statistical analyses and report for working groups and conferences.

Branch members have participated in training sessions for interviewers and other ancillary personnel by explaining procedures for carefully and uniformly collecting data and the relationship of such data to the Study as a whole.

Of the many statistical problems that remain to be solved, the following may be mentioned:

1. The establishment of suitable sampling procedures to insure the inclusion of a certain proportion of repeat pregnancies at each institution.

2. The completion of appropriate study forms, editing procedures, codes and manuals of procedure for each aspect of the Study.

3. The further development of adequate methodological tests of reliability and validity of the data secured.

4. The development of methods to insure a periodic feedback of tabulated data to the institutions.

5. Further planning for data analyses.

The Branch Chief presented a paper on "Some Problems for the Statistician in Collaborative Research" at the December 1959 meeting of the Bio-Statistics Division of the American Statistical Association (New York Area Chapter), New York, New York.

The accomplishments given above relate to the intensive phase of the Collaborative Project. In view of the possibility that the incidence of certain neonatal deficits is so low that even 40,000 live births will not yield enough cases to demonstrate statistical significance, an extensive phase has been postulated. In this phase a number of approaches are envisioned whereby the cases available for study in given categories may be increased. For instance, it is expected that in those communities in which collaborating institutions are located attempts will be made to relate respectively, during the course of the Collaborative Project, the occurrence of neurological disorders to prenatal, labor, and delivery data available from hospital records, vital statistics records, etc. Although the number of cases will be increased, the data available will not be as comprehensive nor as detailed as that in the intensive phase. Branch members have met as a "working group" with the members of the staff of the National Office of Vital Statistics. This group developed proposals dealing with record-anchored studies which may be of value to the extensive phase.

Additional studies, valuable to the conduct of the extensive phase, may be executed by utilizing copies of punch cards of all live births, fetal deaths, and neonatal deaths, purchased by contract or by order from cooperating State and local health departments for the cities concerned with the intensive phase. Such a study of fetal wastage in New York City is currently under way on a joint basis with the New York City Health Department. It is concerned with the tabulations and analyses of approximately 380,000 punch cards for the period 1955-56. It is expected that a number of valuable leads will come to light as a result of this investigation. Additionally, punch cards have been secured for Erie County in New York State, Minneapolis, St. Paul (Minnesota), and Portland (Oregon). Negotiations are under way to secure such data from Baltimore (Maryland), Richmond and adjacent counties (Virginia), and from Philadelphia (Pennsylvania).

Due to a reorganization, effective October 30, 1960, the statisticians, statistical clerks, and other ancillary personnel engaged in certain areas of the Collaborative Project were transferred from the Biometrics Branch to the Perinatal Research Branch. As a result, the Biometrics Branch has no further responsibility in conducting statistical operations, or in supervising or coordinating the statistical activities of the Collaborative Project. This is now a function of the Perinatal Research Branch.

2. Statistical consultation and/or service given to investigators outside of NINDB on other projects.

The following represent the type of consultation and/or service rendered during 1960 to outside investigators on other projects:

(a) Consultation and service to the Employee Health Service, NIH, on an investigation dealing with the use of sick leave, on a research and management basis. A new system for collection and analyses of the "Health Unit Daily Record" was devised.

(b) Consultation given to the Laboratory of Infectious Diseases, NIAID, in connection with a virology study.

(c) Consultation relating to methods of analyses of data in the study of the response of monkeys to asphyxiation, given to an obstetrician engaged in studies of reproduction at the NINDB monkey colony in Puerto Rico.

(d) Consultation with the National Society for Prevention of Blindness, American Foundation for the Blind, Office of Education (HEW), Baltimore City Health Department and the University of Maryland Department of Preventive Medicine, on various possible studies of prevalence, incidence, and etiology of neurological and sensory disorders. Plans are under way to initiate: (1) several retrospective studies dealing with etiology of various neurological disorders, and (2) surveys of the prevalence of serious visual and auditory defects.

(e) A study is being designed jointly with the National Association for Retarded Children to secure information relating to the possible etiology of mental retardation. It is planned to obtain data by mail questionnaire to 65,000 member families of the NARC. It is contemplated that a series of pilot studies and pretests will precede the study proper.

(f) Consultation concerning a retrospective study of etiology of cerebral palsy in Chicago, supported by an NIH grant, with respect to the utilization of proper "normal" controls.

(g) Branch members have worked with the Assistant Commissioner of Health, Research and Planning Section, and the Director, Bureau of Vital Statistics of the Baltimore City Health Department, on a study of smoking and prematurity. Part of this study was financed by contract. The purpose of this study was to determine the incidence of prematurity among offspring of gravidas who smoke and among those who do not smoke, and to include within these groups other co-variables, such as work history, education, blood grouping, and personality characteristics. The final report of this study is now

being prepared for publication. There appears to be evidence on the basis of cigarette smoking histories, obtained prenatally from 2736 Negro women who delivered single live born infants, that the prematurity rate (weight of 2500 grams or less) for mothers who smoked was 18.4% compared with 11.2% for nonsmokers. The incidence of prematurity increased with the amount smoked. This difference was not adversely affected by maternal age, blood group type, initial hemoglobin level, sex of child, working history, education and a psychosomatic complaint score. The evidence presented was generally consistent with that of retrospective studies conducted in California by Simpson and in Birmingham, England, by Lowe.

An evaluation of a series of nutrition studies dealing with "The Underweight Patient as an Increased Obstetric Hazard," "Nutrition and Nutritional Deficiencies as Related to the Premature," and "Epiphyseal Maturation in the Newborn as Related to Maternal Nutritional Status," were rendered at the request of the Director of the Institute. Additionally at his request, a series of papers on "Observations on the Results of Pregnancies in Women Resident in Belfast" were reviewed and evaluated. At the request of personnel in the Perinatal Research Branch, a research grant application designed to develop behavioral tests to aid in the inference of brain damage in the child of early school age, was reviewed in order to determine the adequacy of the study design. At the request of the Epidemiology Branch a paper entitled "Mortality from Cerebrovascular Accidents" was reviewed.

In addition, a number of proposed projects to be submitted for research grants in connection with the so-called "extensive" phase of the Collaborative Project were reviewed in order to evaluate their study designs and merits. These projects were titled: "Epidemiologic Study of Reproductive Wastage," and "Study of Prematurity."

Review of a manuscript entitled "Racial Differences in Visual Acuity of American Youth" was requested by Public Health Reports prior to acceptance for publication. Similarly, at the request of a staff member of the National Institute of Mental Health, a manuscript entitled "An Etiological Re-Classification of Epilepsy and Its Relation to Mental Deficiency" was reviewed prior to consideration for acceptance for publication by the American Journal of Mental Deficiency.

Branch staff member attended a meeting of the Subcommittee on Records of the Committee on Maternal and Child Care of the American Medical Association in December 1959, in New York City, to determine ways in which the Collaborative Project might be of help to the Subcommittee in the development of suitable forms dealing with the prenatal, labor and delivery periods, which might be recommended as standard for hospital use.

Members of the Branch staff have served or are presently serving on the following committees, thus being, in effect, channels of communication whereby problem areas in the neurological field become known to the Branch and whereby the Branch's field of interest and activity become known to other investigators:

(a) Statistical Advisory Committee to "A Study of Use of Statistics on Maternity and Newborn Infant Care in Hospitals."

(b) Advisory Committee on Epidemiology and Biometry (NINDB-liaison).

(c) Committee on Nomenclature and Biometrics of the American Academy of Neurology.

(d) Ad Hoc Committee on Mental Retardation of NIMH.

(e) NIH Advisory Committee to National Health Survey (NINDB-liaison).

(f) Panel for the Study Group on Guide Material for Comparable Studies on Maternal and Perinatal Events Reported on Vital Records of the Public Health Conference on Records and Statistics.

(g) Panel for the Study Group on Development of Working Relationship Between Universities and Statistical Programs and Research in Government of the Public Health Conference on Records and Statistics.

3. Statistical consultation and/or service furnished to clinic and basic research investigators at NINDB in the areas of neurology and blindness.

Various units of NINDB were provided with consultative and statistical services in the design, interpretation, analysis, and evaluation of experiments, including mathematical assistance in development of theoretical models of biological and psychological behavior.

Below are indicated the units in NINDB that received aid from the Biometrics Branch in 1960 and the problems involved:

(a) Laboratory of Neuroanatomical Sciences

Consultation on nerve counts data with respect to the vestibular and cochlear nerves.

(b) Medical Neurology Branch

Statistical assistance in providing a physical method (Archimedes Principle) and a mathematical method (volumetric calculations) of determining the volume of the pituitary gland. Tests of differences between X-ray volumes and actual pituitary volumes were computed. A mathematical model for obtaining a correction factor for estimating the actual pituitary volume from the X-ray volume was devised. Correlations, linear regressions, tests of significance, and graphs were prepared to show relationship between the volume of the

sella and such factors as age, sex, height, weight, volume of the skull and thickness of the calvaria.

Consultation and statistical assistance with reference to four approaches to bio-assay.

(c) Surgical Neurology Branch

Consultation with reference to determining differences between plots on "brain maps."

Consultation on methods of testing differences between operative results and duration of seizures of epilepsy.

Consultation on methodology of determining differences between results of psychological tests.

(d) Ophthalmology Branch

Statistical assistance dealing with regression relationships and their statistical significance, between intraocular pressure and venous pressure of the femoral artery, ophthalmic artery, and iris artery before and after drugs.

Consultation and assistance in the computation of a "modified life table" derived from published data dealing with retinal detachments. Also computation of various statistical parameters, chi-square tests and analysis of variance from tonograph readings.

(e) Collaborative and Field Research

Statistical analysis of (1) gene frequencies from blood test data, and (2) of serum protein surveys.

(f) Epidemiology Branch

Joint study of world-wide mortality from neurological and related disorders. Included in the study are more than 25 diseases of the nervous system. Data, suitable for analysis by age-adjusted rates, for some or all of the diseases of interest are presently available from over 30 countries. Included among the more than 40 countries from which data were sought, were those from which it was believed the desired statistics could be made available. Additional countries were contacted because of their geographic locations and ethnic compositions, even though it was thought unlikely that the requested information could be provided in adequate detail. It is anticipated that this study will be presented at the 1961 meeting of the World Federation of Neurology in Rome.

Consultation regarding sampling and other aspects of a proposed survey in Guam of the prevalence of amyotrophic lateral sclerosis.

B. PROPOSED OBJECTIVES

To date, the energies of the Branch have been directed to the aspects of the program mentioned above. In view of the reorganization mentioned above, the Branch will no longer have any responsibility for the statistical supervision or coordination of the different facets of the Collaborative Project. The reorganization has resulted in the transfer of 9 professional statistical personnel and 29 nonprofessional statistical and typing personnel from the Biometrics Branch to the Perinatal Research Branch which will, henceforth, have the above-mentioned responsibility. The transfer has left the Biometrics Branch with 3 professional statistical personnel and 6 nonprofessional statistical, typing and stenographic personnel. The Branch will in the future devote its effort to stimulating research in studies of prevalence, incidence and etiology of the neurological and sensory organ disorders by assisting in formulating objectives, study designs, data collection and analysis procedures, and by participating, wherever possible, in the analysis and interpretation of the data. The Branch will thus act in a coordinating and catalytic capacity to assist in research areas in which, to date, much remains to be done. The reception accorded Branch contacts for this purpose by official and unofficial agencies has been most gratifying and augurs well for the future. It is believed that the prestige and influence of the NINDB will be enhanced greatly by such mutual cooperative research endeavors. The Branch will continue to furnish consultation and service, upon request, to clinical and basic research scientists in neurology and blindness at the Clinical Center, as well as to other branches of the NINDB.

C. RECRUITMENT

Budget for fiscal 1961 includes 18 positions (7 professional statistical positions and 11 nonprofessional clerical-stenographic positions). This means that 9 positions (4 professional and 5 nonprofessional) are vacant. Recruitment is under way to fill these positions with the best available candidates. This effort will continue relentlessly.

ANNUAL REPORT
Calendar Year 1960
Epidemiology Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

The activities of this Branch during 1960 have included a variety of genetic and epidemiologic projects at the clinical level, among isolated populations and on a broad international scale.

The most productive aspect of our research continues in Guam which is a treasure house of epidemiologic and genetic information. During the past year the significant observations reported in the publications by Hirano, et al., on the intracytoplasmic inclusion bodies and neurofibrillary changes associated with the exceedingly prevalent dementia-Parkinsonism syndrome and amyotrophic lateral sclerosis, has resulted in a new series of studies to evaluate a possible infectious etiology of these disorders on Guam and to study the interrelationship of chronic progressive neurological disorders of unknown cause, namely the "presenile" dementias, Parkinsonism and motor neurone disease. Opportunities for intensive research in diabetes, gout and diaphyseal aclasia are present among the Chamorros, but proceed slowly because of lack of a suitable research facility and personnel on Guam.

Because of the lack of laboratory space at NIH, it was necessary to assign Dr. Hirano to the Department of Neuropathology, Montefiore Hospital, New York, where he will conduct his comparative pathological study of Guam and "Stateside" Parkinsonism and amyotrophic lateral sclerosis. Dr. Simmons Lessell, Dr. Hirano's replacement on Guam, has already demonstrated his ability to conduct independent clinical research in convulsive disorders and the neurological complications of diabetes, as well as to procure needed necropsy specimens and clinical and genetic follow-up data for the numerous studies which the Branch conducts in Micronesia. The new Director of the Guam Department of Medical Services, appointed in mid-1960, has renewed the excellent relationship which our research group has had with the Guam administration.

On the local scene, Dr. Krooth has continued his laboratory investigations of the applications of tissue culture techniques to selected genetic diseases such as galactosemia. Tissue culture is a new and promising area and the observation of the behavior of cells from patients with genetic disease may have far-reaching implications in the elucidation of metabolic pathways and, ultimately, in the treatment of these diseases. Dr. Krooth has also collaborated with Dr. Hin Tjio, NIAMD, in investigating possible chromosomal abberations in human disease. No laboratory space is available for Dr. Krooth from NINDB; his work is being carried out in laboratory space loaned by NIAID.

Dr. Myrianthopoulos has continued his field and laboratory investigations on the genetics and epidemiology of neurologic disorders. The first phase of one study suggests that the frequency of Parkinson's disease is higher among the relatives of patients who develop extrapyramidal symptoms from ataraxic drugs than among the relatives of controls. In a study of conduction velocity time in peroneal muscular atrophy patients, there are peculiar electromyographic patterns which indicate that the lesion in this disease complex may be located either in the nerve, the roots, or the anterior horn cells in affected members of the same family. The collection of material for the blood group typing in amyotrophic lateral sclerosis patients nears completion, and an analysis will be made in 1961. A study of population genetics of Tay-Sachs disease is nearing completion, and a clinical and genetic study of congenital anomalies associated with myotonic dystrophy is continuing.

The study of the epidemiology of a neurological disorder in Minamata, Japan, mentioned in last year's annual report has been published. Investigations in Japanese and United States vinyl chloride plants have revealed a difference in processing which accounts for the intoxication by mercury compound of some of the population in Minamata through the ingestion of poisoned fish. The identification of the mercury toxin was first made in Japan; it was delayed for over a year at N.I.H. because of the lack of a suitable laboratory at NINDB and was finally carried out at the Sanitary Engineering Center (S.E.C.), PHS, Cincinnati, Ohio, when it could conveniently be fitted into the Center's schedule. Further studies are under way by the S.E.C. laboratory to ascertain the possible risk from pollution of the air by mercury in the distillation processes of vinyl chloride in the United States.

A project on the thallium poisoning epidemic which occurred in Southeastern Texas from 1955 to 1959 is still under way and an effort is being made to determine long-term sequelae of this known toxic agent.

The epidemiology of multiple sclerosis is still actively studied by this Branch; consultation in research methods is provided to foreign investigators in Japan, Iceland, Australia, New Zealand, Poland and other countries where such research is being pursued. Dr. Milton Alter who has completed a comparative study of multiple sclerosis in Charleston and Halifax and a study of the incidence of central nervous system malformations in Charleston, is currently investigating the prevalence of multiple sclerosis among ethnic groups in Israel. Dr. Fons is studying the incidence of multiple sclerosis in American Indians in the upper mid-West and in the Southwestern States. It was not feasible for him to complete his study of the cerebral vascular diseases in Rochester, Minnesota.

Dr. Richard Darrell, on assignment from the Communicable Disease Center, is aiding in the study of the incidence and geographic distribution of uveitis, which is being pursued through the records of the Mayo Clinic.

In addition, Dr. Darrell is planning a prospective survey of uveitis in other communities and a project on the epidemiology of congenital strabismus and of myopia among the school children of the Washington suburban area.

The Chief of the Branch participated in several of the aforementioned projects and has continued the study with the National Research Council and the Veterans Administration on the natural history of retrobulbar neuritis and multiple sclerosis.

The mounting emphasis in the Public Health Service on international research has led to an increased effort to clarify many questions in the geographic distribution of diseases of the nervous system. A world-wide mortality study of neurological diseases was initiated with the Biometrics Branch. There has been considerable activity in the newly-organized World Federation of Neurology (WFN). The Branch Chief is Chairman of the Biometry and Genetics Commission of WFN, has been active on program arrangements for the symposium on "The Contributions from Geographic Isolates to Epidemiology and Genetics in Neurology" at the forthcoming International Congress of Neurology; he is also concerned with the program of the forthcoming conference of the International Epidemiologic Association. It has been possible for the Branch Chief to integrate much of his international research activities and experience with his duties as the Institute's representative to NIH in connection with the Public Law 480 program and for the deliberations of the Task Force for Off-Site Installations.

It has been difficult to launch several new programs because of the lack of suitable space for essential supportive laboratory work at NIH and the disruption caused by the dispersion of personnel resulting from our unsatisfactory space assignments away from NIH. This Branch has been moved about or off the NIH reservation 9 times in less than 5 years; this practice places us under a tremendous handicap in fulfilling our obligations to the Institute, and has made almost impossible the recruitment of the scarce and highly specialized professional staff required for the Branch. This lack of a suitable space allotment has also jeopardized the final organization of the proposed Sections on Human Genetics, Environmental and Geographic Studies, and Animal Genetics and Epizotiology.

This Branch intends to carry out fully its unique responsibilities both within the NIH and in international research; prompt action on its critical space requirement will insure this in the coming fiscal year.

PUBLICATIONS

Published or in Press

1. Myrianthopoulos, N. C., and Rowley, P. T.: Monozygotic Twins Concordant for Huntington's Chorea. Neurology 10:506, 1960

2. Myrianthopoulos, N. C., and Mackay, R. P.: Multiple Sclerosis in Twins and Their Relatives: Genetic Analysis of Family Histories. Acta Gen. Stat. Med. 10:33, 1960

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5. Krooth, R. S. and Weinberg, A. (1960): Properties of Galactosemic Cells in Culture. Biochem. Biophys. Res. Comm., in press.

6. Alter, Milton; Talbert, O. Rhett; Allison, R. S.; Kurland, Leonard T.: The Geographic Distribution of Multiple Sclerosis. A Comparative Study in Charleston County, South Carolina and Halifax County, Nova Scotia. I. Prevalence in Charleston County, South Carolina. Journal of the South Carolina Medical Association, 56:209, 1960

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9. Okinaka, Shigeo; McAlpine, Douglas; Miyagawa, Kuheita; Suwa, Nozomi; Kuroiwa, Yoshigoro; Shiraki, Hirotsugu; Araki, Shukuro; and Kurland, Leonard T.: Multiple Sclerosis in Northern and Southern Japan. World Neurology 1:1, 1960.

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14. Hirano, Asao; Krooth, Robert S.; Kurland, Leonard T.: Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam. 1. Clinical Features, in press, Brain.

15. Hirano, Asao; Malamud, Nathan and Kurland, Leonard T.: Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam. II. Pathological Study, in press, Brain.

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*18. Fine, David I. M.; Barron, Kevin D.; and Hirano, Asao: Central Nervous System Lipidosis in an Adult with Atrophy of the Cerebellar Granular Layer. Journal of Neuropathology and Experimental Neurology 19:3, 1960.

*19. Friedman, Arnold P.; Carton, Charles A.; Hirano, Asao: Facial Pain. Postgraduate Medicine 27:6, 1960.

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*20. Friedman, A. P.; Carton, Charles A.; and Hirano, Asao: Cyclical Head and Face Pain. AMA Archives of Neurology 2:1, 1960

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*Papers 18 thru 21 were initiated by Dr. Hirano prior to his joining this Branch.

REPORTS (Unpublished data)

I. Myrianthopoulos, N. C., Kurland, L. T., and Kurland, A.: A Genetic Study of Parkinsonism Produced by Ataraxic Drugs.

II. Menkes, John H., and Alter, Milton: A New, Possibly Sex-Linked Recessive Disorder with Growth Retardation, Puculiar Hair (Phili Torti) and Severe Mental and motor impairment. Paper presented at the American Academy of Neurology, April 1960.

ELECTED TO MEMBERSHIP

International Epidemiological Association, May, 1960. Dr. Kurland

1960 - Clinical Professor of Neurology, Howard University, Dr. Kurland

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Clinical Study of Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam

Principal Investigator: Asao Hirano

Other Investigators: Robert S. Krooth
Leonard T. Kurland

Man-Years: Total - 1-1/2
Professional - 1/2
Other - 1

Project Description:

This is a clinical study of a neurological disorder manifested by Parkinsonian syndrome with mental deterioration found among the indigenous Chamorros of Guam. It affects Chamorro adults in the fourth to sixth decades, and death usually results about three to five years after the recognized onset. The onset is insidious and the main clinical features are mental deterioration of the organic type (dementia) and the Parkinsonian syndrome. In addition, signs of upper motor neurone involvement or the ALS syndrome are observed in about 1/3 of the cases. Many patients have a family history of ALS, "Parkinsonism", or both.

Although the disease may demonstrate the cardinal features of mental deterioration, extrapyramidal disease and motor neurone disease of the Jakob-Creutzfeldt disease, it cannot at present be considered as analagous to any one of the numerous clinical variants included in this grouping. There are also indications that this disorder on Guam is not of a postencephalitic nature. Pathological and other studies now under way are expected to clarify these matters.

PART B.

Paper to be published in Brain, 1961.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Pathological Study of Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam

Principal Investigators: Asao Hirano
Leonard T. Kurland

Other Investigator: Nathan Malamud, Professor of Psychiatry and Neuropathology, University of California, San Francisco, California.

Man-Years: Total - 1-1/2
Professional - 1/2
Other - 1

Project Description:

This is a pathologic study of seventeen cases of a neurological disorder manifested by a Parkinsonian syndrome with mental deterioration found among the Chamorros of Guam. The gross neuropathological features are the presence of cortical atrophy, and depigmentation of the substantia nigra and locus caeruleus. The consistent finding on microscopic examination is wide-spread ganglion cell degeneration of the central nervous system. It affects primarily the cortex of the frontal and temporal lobes, Ammon's horn, amygdaloid nucleus, hypothalamus, globus pallidus, thalamus, periaqueductal structures, substantia nigra and tegmentum of the brain stem. There are numerous intra-ganglionic fibrillary changes and scattered intracytoplasmic granulovacuolar bodies in the affected neurones of these areas. However, typical senile plaques are not found in any of the cases. In addition, there is a diffuse loss of ganglion cells of a variable degree, most marked in the globus pallidus and substantia nigra. Reactive glial proliferation accompanies the neuronal involvement. Myelin sheaths are usually spared with the exception of the cases which presented clinically with features of amyotrophic lateral sclerosis and pathologically with degeneration of motor neurones, as well as bilateral demyelination of the pyramidal tracts. Further studies on the relationship of this disorder to ALS and efforts to determine the course of this disorder continue.

PART B.

Manuscript to be published in Brain.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Investigations of Chamorros on the Island of Guam

Principal Investigators: Leonard T. Kurland
Asao Hirano
Simmons Lessell
Jose Torres
Nathan Malamud
Leon Rosen

Other Investigators: Joseph Kovacs, Director
Department of Medical Services
Territory of Guam

Man-Years: Total - 4
Professional - 2
Other - 2

Project Description:

The stable population, small size of the Island, high quality of available medical care, large sibships and high incidence of certain chronic diseases amongst the native population make Guam a valuable natural epidemiological laboratory. Investigations of amyotrophic lateral sclerosis are still in progress. In addition, efforts are underway to define the characteristics of the presenile-dementia-extrapyramidal syndrome discovered to be so prevalent on Guam. Simple goiter appears to be unduly frequent on the Island, a finding which gains even greater significance in light of the high iodine content of the native diet. Diabetes mellitus appears to be excessively prevalent amongst the Chamorros and a survey of neurological illness in this subgroup has been completed. Hyperurecemia is also very frequent amongst the Chamorros. Finally, seizure disorders on Guam have come under the scrutiny of this Branch because of their alleged frequency and because epidemiological data regarding seizure disorders are not presently available for tropical areas.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Seizure Disorders

Principal Investigators: Simmons Lessell
Leonard T. Kurland

Other Investigator: Jose Torres

Man-Years: Total
Professional
Other

Project Description:

Physicians working on Guam have been aware for some time of an apparently high incidence of febrile seizures amongst the Chamorros children. A survey was undertaken in a typical Guamanian village using the public grammar school children as index cases. The prevalence of positive seizure histories in the children and their siblings was found to be 9.6/100. Only four of the 78 individuals with positive seizure histories had ever experienced an afebrile seizure. A significantly higher prevalence of positive histories was obtained from the parents and siblings of "affected" index cases than from the parents and siblings of "non-affected" individuals. Further studies are under way.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Neurological Disease in Diabetes Mellitus Patients

Principal Investigators: Simmons Lessell
Leonard T. Kurland

Other Investigators: Jose Torres
Joseph Kovacs, Director
Department of Medical Services
Territory of Guam

Project Description:

A survey of Chamorro adults completed in the latter part of 1959 revealed a prevalence of 12.6/100 for diabetes. A random segment of this diabetic population was selected for a study to determine the incidence of neurological disease amongst Chamorro diabetics. One hundred such individuals were subjected to a neurological interview and examination. Five cases of the Presenile-Dementia Extrapyramidal Syndrome were encountered. Five cases of cerebral vascular disease were encountered in the group and 8 instances of diabetic peripheral neuropathy were noted. Three patients had suffered a Bell's Palsy. Further work on the relationship of diabetes to the neurological disorders is contemplated.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Serological Survey Among Guamanians

Principal Investigator: Simmons Lessell

Other Investigator: Leonard T. Kurland

Collaborating Investigators: (To be designated)

Project Description:

The histopathologic findings of neurofibrillary tangles and intracytoplasmic granulovacuolar bodies in the central nervous system of patients dying with the dementia-Parkinsonism and ALS syndrome on Guam again raises the question of a viral etiology.

Dr. Lessell is collecting sera from patients and the unaffected population. At a laboratory to be selected titers will be measured for antibodies against arbor and other viruses, especially those occurring predominantly in tropical areas.

This survey will be extended to include the Chamorro population on Rota and Saipan and the Carolinians of Saipan and one of the Caroline Islands.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Relation of Ability to Taste Phenylthiourea to Thyroid Disease

Principal Investigator: Robert S. Krooth

Other Investigators: Dr. T. E. Ross, NIAMD
Dr. Bernard Witkopf, NIAMD

Man-Years: Total - 3/12
Professional - 3/12

Project Description:

As noted, in detail in last year's report, the association of certain types of thyroid disease with ability to taste phenylthiourea (and related compounds) has been observed by several groups of investigators. Evidence bearing on this association is being gradually accumulated, but our series is too small at present to permit a meaningful statistical analysis.

The studies, carried on in collaboration with Dr. Witkopf, on the chemical nucleus conferring taste activity have thus far led to no clear conclusions. The two difficulties have been the low solubility of the compounds in usual organic solvents, and the difficulty in determining how much activity a particular compound has.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Karyotypic Abnormalities in Human Disease

Principal Investigator: Hin Tjio, NIAMD

Collaborating Investigator: Robert S. Krooth

Man-Years: Total - 3/12
Professional - 3/12

Project Description:

A search is being made in our laboratory, as in others, for new chromosomal abnormalities in human disease. Cell lines have been developed from biopsies on 5 patients with congenital malformations, in each case of a kind not previously reported. In every instance thus far the chromosomes have been normal in morphology and number.

Certain other studies on the human chromosomes are being carried out.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Application of the Technique of Tissue Culture to Selected Genetics Diseases

Principal Investigator: Robert S. Krooth

Collaborating Investigators: Dr. Arnold W. Weinberg, NIAMD
Dr. Paul Altrocci, NINDB
Dr. Harry S. Eagle, NIAID

Other Investigators: Dr. Herman S. Kalckar, Department of Biochemistry
Johns Hopkins University
Dr. Howard B. Hamilton, U.S. Atomic Bomb Casualty Commission, Hiroshima, Japan

Man-Years: Total - 6/12
Professional - 6/12

Project Description:

Studies to follow a number of genetic diseases in tissue culture are being continued. Biopsies are performed on affected patients, and cell lines are then developed from the excised tissue. Evidence bearing on the persistence of the defect in cell lines from affected persons (compared with cell lines from controls) is obtained. Ultimately, mutant cell lines from patients with inborn metabolic errors should prove useful in the study of genetic exchange among human cells as well as in other aspects. The system permits one to study mutant human cells in a way which hitherto could be applied only to mutants of bacteria and fungi. However, the number of genetic diseases which are susceptible to this approach is at present quite limited.

Galactosemia has been shown to persist as defect in culture, even after 6 months of propagation and an increase in cell number known to exceed 30-billion fold. The defect can be shown by growth studies, the ability of the cells to metabolize galactose-1-C^{14}, and direct enzymatic assays. The galactosemic cells show a pattern of galactose

sensitivity similar to the transferaseless mutants of E. coli. In each case the galactosemic cells are compared with control ones. Corresponding data have been obtained for one heterozygote, and here also the defect persists.

Wilson's disease and hypocatalasemia are also under study by this approach.

Our efforts to follow Gaucher's disease and certain other histiocytic states in culture (referred to in the last report) have not yet succeeded. Indefinite propagation of recognizeably affected cells has not been achieved although the cells will attach to glass, spread, and appear (at least) to increase in number for a few weeks. Thereafter, though the cells can be made to survive for months, no evidence of growth is found.

PART B.

Krooth, R. S., and Weinberg, A. (1960) Properties of Galactosemic Cells in Culture. Biochem. Biophys. Research. Comm. (in press)

PHS - NIH
Individual Project Report
Calendar Year - 1960

PART A.

Project Title: The Outbreak of a Neurological Disorder in Minamata, Japan and Its Relationship to the Ingestion of seafood Contaminated by Mercury Compounds

Principal Investigator: Leonard T. Kurland

Other Investigators: Stanley N. Faro, Howard D. Seidler

Collaborating Institutions: Kumamoto University School of Medicine
Tokyo University, Department of Neuropathology, Sanitary Engineering Center, PHS, Cincinnati, Ohio

Project Description:

An epidemic of a chronic progressive and often fatal neurological disorder occurring in families residing in fishing villages near Minamata Bay has been under study by Japanese scientists since 1953. Pathological examination of central nervous system specimens suggested a similarity to organic mercury poisoning. Mercury in high concentration was found in marine life from Minamata Bay and from mud especially near the effluent channel of a large plant manufacturing vinyl chloride. Mercuric chloride catalyst is lost in appreciable quantities from this plant. Clinical and laboratory studies here have confirmed these findings. Studies in the vicinity of U. S. vinyl chloride plants revealed that a difference in the purification process prevented undue contamination of nearby waters although air pollution with mercury had to be guarded against. Recommendations have been made to reduce the threat of continued outbreaks in the vicinity of the Japanese plants.

PART B.

Kurland, Leonard T.; Faro, Stanley, and Siedler, Howard D. Minamata Disease. The Outbreak of a Neurological Disorder in Minamata, Japan and its Relationship to the Ingestion of Seafood Contaminated by Mercury Compounds, World Neurology 1:5, 1960

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Epidemiologic Investigation of Outbreak of Thallium Poisoning in Southeastern Texas, 1955-59

Principal Investigator: Stanley N. Faro

Other Investigators: Anthony L. Fons
Leonard T. Kurland
James W. Crawley
Samuel J. L. Pieper

Project Description:

An outbreak of Thallotoxicosis occurred in Southeastern Texas from January 1955 through December 1959; 107 children through age 14 were hospitalized during this 4-year period for accidental ingestion of thallium sulfate-containing rodenticides and insecticides. Sixty of these are known to have ingested one particular roach poison (Echols). There is reason to believe that many more children were seen in outpatient clinics and private offices and were not hospitalized.

Roaches and ants are prevalent in the humid gulf coast region of Texas and numerous compounds have been manufactured by exterminators in an effort to rid the area of these pests. In the late '40's exterminators in Kingsville, Texas were using a mixture of thallium sulfate, cereal and syrup as a rodenticide. Two deaths were reported in 1948 among 4 children who ate this mixture as "candy". In 1952 The Echols Mfg. Co. of Corpus Christie bought the business of one of the Kingsville Exterminating Companies and began the manufacture of a rodenticide containing 3% thallium sulfate in a palatable vanilla wafer base.

During the early part of 1957 the legislature of the State of Texas enacted legislation, enforced by the Division of Food and Drugs of the State Department of Health in Austin, Texas, restricting the manufacture of any mixture containing thallium sulfate or any thallium salt to contain not more than 1% of thallium. The study includes a

review of clinical and pathological features, biochemical effects, and long-term follow up for neurological and other sequelae.

PART B.

A report is being prepared.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Epidemiologic Investigation of Fish Poisoning in the South Pacific

Principal Investigator: Albert H. Banner, Director
Hawaii Marine Laboratory; Philip Helfrisch,
Assistant Marine Biologist, Hawaii Marine Lab.

Co-Investigator: Leonard T. Kurland

Man-Years: Total - 1
Professional - 1/2
Other - 1/2

Project Description:

Poisoning from the ingestion of fish occurs in many areas of the world. Little is known of the specific toxic agent or the reasons for its sporadic and often unpredictable development. There has apparently occurred an increase in the incidence of fish poisoning with its variety of neurological symptoms in the Western and South Pacific area. It is of sufficient magnitude to warrant further investigation to delineate more exactly the nature of fish poisoning and to obtain an estimate of the morbidity and mortality following ingestion of such fish.

To facilitate the epidemiologic study, the Branch is assisting the above-named investigators in the preparation of the study design, the drafting of a pamphlet to guide medical practitioners and physicians in Oceania participating in the study and the drafting of a questionnaire designed to ascertain the fundamental epidemiologic characteristics of fish poisoning. As necessary and where possible, when severe outbreaks of fish poisoning occur, competent medical staff, including neurologists when possible, and biologists will visit the scene to investigate the outbreak and to aid the local authorities.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Does Multiple Sclerosis Exist Among American Indians?

Principal Investigator: Anthony Fons

Collaborating Investigator: Leonard T. Kurland

Man-Years: Total - 1/8
Professional - 1/8

Project Description:

There has been no published material containing epidemiological data on the frequency and distribution of multiple sclerosis or other demyelinating diseases among the American Indians. The Indian population resides in areas that vary widely in climate, geography and natural resources.

The relatively recent introduction of a cross-indexing system in the PHS medical care installations has greatly facilitated the review of records for specific diagnoses. The record systems of three Indian Reservations in North and South Dakota were reviewed recently in an attempt to establish the absence or presence of possible cases of multiple sclerosis.

Because of this opportunity to remedy the paucity of information on M.S. in this ethnic group living in varied socio-economic, geographic, and environmental conditions with high morbidity and mortality rates from infectious disease, exploration of an extension of the "pilot" three-Reservation survey to include other Indian populations is currently being undertaken.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The incidence of Anencephalus, Hydrocephalus and Spina Bifida in Charleston County, S. C., 1946-1955

Principal Investigator: Milton Alter

Man-Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

Anencephalus, hydrocephalus, and spina bifida represent the major types of congenital malformations of the central nervous system. A survey of hospital mortality, clinic and physicians' records in Charleston County, South Carolina for the period 1946-1955 disclosed 140 cases with these malformations

The incidence of anencephalus, hydrocephalus, and spina bifida per 1000 live births was 0.53, 0.96, and 0.71 respectively. Anencephalus and spina bifida is less common in negroes than whites, but hydrocephalus is equally frequent in the two races. Anencephalus and spina bifida are more common in females. Hydrocephalus is more common in males. The frequency of anencephalus is correlated with maternal age. Familial aggregation of cases was not observed except for an instance of anencephalus in twin negro girls. No seasonal or secular trend in incidence was noted.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Prevalence of Multiple Sclerosis Among Ethnic Groups in Israel

Principal Investigator: Milton Alter

Other Investigators: L. Halpern, Hadassah University Hospital
J. Silberstein, Israeli Ministry of Health
L. Bornstein, Israeli Ministry of Health

Cooperating Units: Hadassah University Hospitals
Israeli Ministry of Health
Kuppat Cholim Medical Organization

Man-Years: Total - 2
Professional - 1
Other - 1

Project Description:

The highly diverse population of Israel, consisting of elements drawn from many parts of the world offers a unique opportunity to determine the relative prevalence of Multiple Sclerosis in various ethnic groups. Intensive efforts are being made to locate and examine diagnosed cases of Multiple Sclerosis and clinically similar disorders.

Clinical impressions among neurologists working in the country many years, indicate that relatively few cases occur among Jewish immigrants from Middle Eastern countries and native-born Jews while ethnic groups from Northern and Western Europe are relatively frequently affected.

Preliminary results tend to confirm this impression with a single exception, i.e., Jews born in Iran (Persia) seem to have an unusually high prevalence of Multiple Sclerosis, although recent information suggests that the Jews in Israel from Persia had been born in Eastern Europe and had migrated to Persia in the early part of the century. If the clinical impression regarding the relative prevalence of Multiple Sclerosis among ethnic groups can be confirmed, studies of dietary differences, life patterns, genetic factors, immunological responses and other appropriate investigations will be initiated among samples of the affected individuals and suitable controls in an effort to account for the prevalence difference.

PART B.
None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Multiple Sclerosis Among American Negroes

Principal Investigator: Milton Alter

Cooperating Units: Neurological Institute, New York

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

There is some evidence that hereditary factors may influence the occurrence of Multiple Sclerosis. It follows that certain races may show a relative resistance to this disease or that the disease may assume atypical forms in certain races.

There is little information on the occurrence of Multiple Sclerosis among Negroes. If this race were relatively resistant to the disease or manifested atypical forms, the relatively low prevalence of Multiple Sclerosis in subtropical and tropical areas, largely inhabited by negroes, might be explained.

In an effort to accumulate information on manifestation of M.S. in the Negro race, the records of cases seen at the Neurological Institute of New York between 1947 and 1957 were reviewed. Approximately 100 cases in negroes were found and are being analyzed for clinical characteristics such as age and mode of onset, course, systems affected.

Correlation between the clinical features and length of residence in the North are being made.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Cerebellar Ataxia, Congenital Cataracts, and Retarded Physical and Mental Maturation in a Negro Family. (Marinesco-Sjogren Syndrome)

Principal Investigator: Milton Alter

Man-Years: 1/10

Project Description:

A rare hereditary syndrome characterized by cerebellar ataxia, congenital cataracts and retarded physical and mental maturation was first described by Marinesco in 1937. Since then several additional families have been found and the mode of inheritance characterized as autosomal recessive.

The first family so affected to be identified in America and the first in Negroes is currently being studied. The mode of inheritance may be autosomal dominant in this family.

Biochemical studies on two of the affected individuals are being carried out in an effort to identify an "inborn metabolic error".

PART B.

None

The relation between such factors as maternal age, parity, maternal health, fertility, place of residence, social status and the frequency of congenital malformations of the nervous system were reviewed. Problems in classification of malformations were mentioned.

Possible etiologic factors including heredity, infection, undernutrition, intoxication, climate, allergic reactions were examined in the light of current epidemiologic data.

PART B.

None

PHA - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Epidemiology of Congenital Malformations of the Nervous System with Special Reference to a Survey in Charleston, South Carolina

Principal Investigator: Milton Alter

Man-Years: Total - 2/12

Project Description:

Aspects of the frequency and population selectivity of anencephalus, hydrocephalus and spina bifida in Charleston County, South Carolina, were studied for the period 1946 through 1955.

An incidence of between 2 and 3 per 1000 live births was found for the combination of these malformations. Hydrocephalus was most common (0.94 per 1000 live births); spina bifida was second in frequency (0.69 per 1000 live births); and anencephalus was least common (0.53 per 1000 live births). The association of hydrocephalus and spina bifida was shown not to be fortuitous. The incidence of spina bifida and anencephalus was only half as great in negro infants as in whites, but hydrocephalus occurred with equal frequency in the two races.

A large female preponderance was found in anencephalic infants in both negro and white races. Spina bifida cases showed only a slight female preponderance, whereas among hydrocephalic infants, males predominated.

The data from the Charleston survey were compared with similar data gathered from various parts of the world. Charleston, with respect to the range of frequencies of hydrocephalus and spina bifida, falls rather in the middle but has a relatively low incidence of anencephalus. No secular or seasonal pattern of incidence was discernable in the Charleston data in contrast to reports of a falling incidence of anencephalus and spina bifida reported from other areas and an alleged tendency for anencephalic births to occur in the colder months.

The relation between such factors as maternal age, parity, maternal health, fertility, place of residence, social status and the frequency of congenital malformations of the nervous system were reviewed. Problems in classification of malformations were mentioned.

Possible etiologic factors including heredity, infection, undernutrition, intoxication, climate, allergic reactions were examined in the light of current epidemiologic data.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Prospective Study of the Epidemiology and Geographic Distribution of Uveitis in Three Eastern Communities.

Principal Investigator: Richard W. Darrell

Other Investigator: Leonard T. Kurland

Collaborating Institute: (To be designated)

Man-Years: Total - 1/2
Professional - 1/4
Other - 1/4

Project Description:

The new cases of uveitis occurring during 1961 in the resident populations of three Eastern cities of about 50,000 will be analyzed with respect to characteristics of the patient, of the environment, and of the natural history of the disease.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Retrospective Survey of the Incidence and Geographic Distribution of Uveitis in Rochester, Minnesota

Principal Investigator: Henry P. Wagener, Mayo Clinic, Rochester, Minnesota

Other Investigators: Leonard T. Kurland
Richard W. Darrell

Man-Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

There have been no total population surveys on the incidence, prevalence and distribution of uveitis. These parameters are now being studied through a retrospective study of the Mayo Clinic records of the resident population of Rochester, Minnesota during a 10-year period. Approximately one-half of the records have been abstracted.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Prospective Study of Epidemiology and Geographic Distribution of Congenital Strabismus in Three Eastern Communities

Principal Investigator: Richard W. Darrell

Other Investigator: Leonard T. Kurland

Collaborating Investigator: (To be designated)

Man-Years: Total - 1/2
Professional - 1/4
Other - 1/4

Project Description:

In conjunction with a prospective study of uveitis, new cases of congenital strabismus occurring during 1961 in the resident populations of three Eastern cities will be analyzed epidemiologically.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Association of Blood Groups to Amyotrophic Lateral Sclerosis

Principal Investigator: N. Myrianthopoulos

Other Investigators: P. Schmidt, LBBP
Leonard T. Kurland

Cooperating Units: Laboratory of Blood and Blood Products
Division of Biologic Standards

Man Years: Total - 2/12
Professional - 2/12

Project Description:

A pilot study to determine if there exists any selection for a specific blood type among patients with motor neurone disease.

Present Status: The medical facilities of the Army, Navy, Air Force and Veterans Administration, the Guam Research Center, and many practicing neurologists have been providing blood and saliva specimens as in the past. Collection of specimens will continue for approximately six more months at which time an adequate sample for analysis is expected to have been accumulated.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Genetic and Clinical Investigations in Myotonic Dystrophy

Principal Investigator: N. Myrianthopoulos

Other Investigators: J. Caughey, NINDB (Now at the University of Otago, Dunedin, N.Z.)

Man-Years: Total - 3/12
Professional - 1/12
Other - 2/12

Project Description:

A study based on history and examination to determine if children of patients with myotonic dystrophy show congenital anomalies more frequently than expected; if there exists a significant difference in the occurrence of congenital anomalies between children of parents with the complete syndrome and children of patients who show only cataract; if the sex of the affected parent has any effect in the production of congenital anomalies among the children; and what is the reproductive fitness of persons affected with myotonic dystrophy.

Present Status: Extensive and detailed pedigrees have been obtained for five families in which myotonic dystrophy has occurred. It is felt that at least five more families must be thoroughly worked out before an analysis can be attempted. Since these families are scattered throughout the U.S. and extensive field work is required to obtain the necessary clinical and genetic information, the investigation is practically restricted to the summer months when COSTEP help for field work is available.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Detection of the Genetic Carrier in Peroneal Muscular Atrophy by Conduction Velocity Studies

Principal Investigator: N. Myrianthopoulos

Other Investigators: M. Lane, NINDB
D. Silberberg, NINDB

Man-Years: Total - 3/12
Professional - 1/12
Other - 2/12

Project Description:

A study to determine whether the genetic carrier (whether unaffected children of affected parents or individuals showing lack of penetrance) can be detected by slow peripheral nerve conduction velocity. A slowing of conduction velocity in the peroneal, ulnar and other nerves has been repeatedly demonstrated in patients with peroneal muscular atrophy.

Present Status: About 100 affected, suspected genetic carriers and normal members of four families with peroneal muscular atrophy have been tested for conduction velocity of their anterior tibial and ulnar nerves. The results have not yet been evaluated. In the course of these studies some findings suggested that a distinction may perhaps be drawn between a purely peripheral and a myelopathic form of the disease on the basis of electromyographic findings. It was, therefore, decided to extend the investigations to more individuals in order to confirm this impression.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Detection of the Heterozygote in Cerebroretinal Degeneration (Amaurotic Family Idiocy, Juvenile or Spielmeyer-Vogt Type)

Principal Investigator: N. Myrianthopoulos

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

Examination of a large number of peripheral blood smears from patients with the infantile form of cerebroretinal degeneration, and smears from their parents and siblings, failed to disclose any diagnostic changes in the peripheral blood. Examination of blood smears from one patient with the Juvenile form of the disease disclosed very striking changes in the lymphocytes in the form of large vacuoles in the cytoplasm. Blood smears from eight other patients with the juvenile form, their parents and siblings did not show these changes.

All reports in the literature of vacuolated lymphocytes in this disease come from the Scandinavian countries. Several investigators in the U.S. reported that they failed to observe them in their patients. It is interesting to note that the family of the patient who showed vacuolated lymphocytes emigrated to the U.S. from Sweden. It is possible that we are dealing with a Scandinavian variant of this disease. More cases are now being sought to confirm this hypothesis.

PART B.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Some Epidemiologic Features of Tay-Sachs Disease

Principal Investigator: N. Myrianthopoulos

Man-Years: Total - 3/12
Professional - 2/12
Other - 1/12

Project Description:

A study to determine the incidence and prevalence of Tay-Sachs disease among the Jewish and non-Jewish population of the United States by mortality statistics.

Present Status: Mortality statistics for the years 1954, 1955, 1956 and 1957 have been collected. Information concerning the family histories of the deceased and pertaining to the ethnic origin, religious affiliation and consanguinity of the parents, and the occurrence of the disease among the siblings has been obtained. The data are now being analyzed and a report is being prepared.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Multiple Congential Anomalies in Three Children of a Family

Principal Investigator: N. Myrianthopoulos

Other Investigators: R. Hamburger
C. Cheney

Cooperating Units: Yale University School of Medicine
Grace-New Haven Hospital

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

A genetic, clinical, anatomical and pathological study of what appears to be a new syndrome not previously described and characterized by multiple congenital abnormalities. These have appeared in three successive children of a New England family, all of whom died within one month after birth. A report is being prepared.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Parkinsonism - Ataraxic Drugs Study

Principal Investigator: N. Myrianthopoulos

Other Investigators: Leonard T. Kurland
A. Kurland, Spring Grove State Hospital

Cooperating Unit: Spring Grove State Hospital

Man-Years: Total - 6/12
Professional - 3/12
Other - 3/12

Project Description:

A study to determine the occurrence of Parkinson's disease among the relatives of two groups of patients: Those who show Parkinsonian symptoms on high therepeutic dosages of certain phenothiazine derivatives, and those who prove to be resistant to the side effects of these drugs. The two groups of patients, actually patients and controls, have been selected from the patient population of Spring Grove State Hospital.

Present Status: The study is now completed. Among 728 relatives of 59 patients susceptible to ataraxic drugs, 13 cases of Parkinson's disease were found. Of these, one is a bona fide post-encephaletic case. Among 777 relatives of 67 controls resistant to ataraxic drugs, three cases of Parkinson's disease were found. All three were described by the attending physicians as being of the arteriosclerotic type. Secondary cases of Parkinson's disease were found in two cases (13%), one among the relatives of the patient group and one among the relatives of the control group.

These findings were analysed by breaking them down by age and comparing them to age-adjusted, expected prevalence rates from Kurland's data. In all age groups the observed rate was higher than the expected in the relatives of the patient group by at least a factor of 4 which is significant. It was not different

in the relatives of the patient group. No further interpretation is attempted at this time. The data are being implemented by a similar study at St. Elizabeth's Hospital

PART B.

Myrianthopoulos, N. C., Kurland, L. T. and Kurland, A.: A Genetic Study of Parkinsonism Produced by Ataraxic Drugs. Report presented at a symposium on Parkinson's Disease, Arden House, N. Y., March 1960. Publication plans uncertain.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Survey of Schizophrenics Among the Relatives of Schizophrenia Patients

Principal Investigator: N. Myrianthopoulos

Cooperating Unit: Spring Grove State Hospital
St. Elizabeth's Hospital

Man-Years: Total - 1/12
Professional - 0.5/12
Other - 0.5/12

Project Description:

A study to determine the occurrence of schizophrenia among the relatives of patients who have already been selected for another project (Parkinsonism - ataraxic drugs) and to determine the role of inheritance in Schizophrenia. This project being a by-product of another project, has the advantage of overcoming some of the biases involved in selecting an adequate sample. The collection of data from Spring Grove State Hospital has been completed. These will be implemented with data from St. Elizabeth's Hospital concurrently with the project on ataraxic drugs conducted there before genetic analysis is attempted.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Genetic Study of Parkinsonism and the Dystonias Produced by Ataraxic Drugs

Principal Investigator: N. Myrianthopoulos

Other Investigators: Francis Waldrop

Cooperating Unit: St. Elizabeth's Hospital

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

A study to replicate the conditions and confirm the findings of the Parkinsonism-Ataraxic Drugs study. If the impression is confirmed that there is an hereditary susceptibility to Parkinsonism produced by phenothiazine derivatives then an experimental procedure will be followed to test a genetic hypothesis: Two groups of patients will be selected from St. Elizabeth's Hospital, one susceptible and one resistant to ataraxic drugs. The basis for selection of both groups of patients would be that they have a living relative with a coefficient of relationship of 0.5 (percent of sibling) who is now a patient at St. Elizabeth's Hospital and available for treatment with ataraxic drugs. A selected drug would then be administered to these relatives and their reactions observed and graded. The same procedure will be used with relatives of patients who show severe dystonia when given ataraxic drugs. The known degree of relationship of relative to patient will serve as a basis for prediction of the eventual outcome if susceptibility to ataraxic drugs is inherited along Mendelian lines.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Epidemiology and Genetics of Hydrocephalus

Principal Investigator: N. Myrianthopoulos

Other Investigators: L. T. Kurland

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

A synthesis and interpretation of the available data on the subject. The work of various investigators regarding the etiology, pathogenesis, geographic distribution, population and sex selectivity, relation to other CNS defects and genetic background of hydrocephalus has been critically reviewed and certain interpretations and recommendations made. They were presented at the annual meeting of the Houston Neurological Society, Houston, Texas, in March, 1960.

PART B.

Myrianthopoulos, N. C., and Kurland, L. T.,

Present Concepts of the Epidemiology and Genetics of Hydrocephalus. Houston Neurological Society Symposia, Charles Thomas, Publisher, in press.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: International Mortality Study

Principal Investigator: Irving Goldberg

Other Investigator: Leonard T. Kurland

Man-Years: Total - 3/12
Professional - 3/12

Project Description:

This is a study of worldwide mortality from more than 25 neurological or related disorders. Material suitable for analysis for age adjusted rates for some or all of the diseases of interest and presently available for all 30 countries. Data were sought from those countries from which it was believed the desired statistics could be made available. Additional countries were contacted because of their geographic locations and ethnic compositions even though it was thought unlikely that the requested information could be provided in adequate detail. It is anticipated that an analysis of the material will be completed in time for presentation at the World Federation of Neurology meeting in September, 1961, because of its international scope.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Effect of Climate and Other Environmental Factors in the Progress of Multiple Sclerosis. One Aspect of a Broader Investigation of the Natural History of Multiple Sclerosis

Principal Investigator: Leonard T. Kurland

Other Investigators: Gilbert Beebe and Dean Nefzger, Follow-up Agency, National Research Council, Washington, D. C.
J. F. Kurtzke, Chief, Neurology Service, V. A. Hospital, Coatesville, Pennsylvania
Thomas Auth, Neurology Dept., Veterans' Administration, Washington, D. C.

Cooperating Units: Follow-up Agency, NRC, Washington, D. C.
Veterans' Administration, Washington, D. C.

Man-Years: Total - 3/12
Professional - 2/12
Other - 1/12

Project Description:

Patients diagnosed as having had retrobulbar neuritis, 1942-1945, are now being examined by neurologists. Preliminary results indicate a low frequency (about 10%) Multiple Sclerosis, as contrasted to 45-50% expected on the basis of available data in medical literature.

PART B.

None

ANNUAL REPORT
Calendar Year 1960
Epidemiology Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

The activities of this Branch during 1960 have included a variety of genetic and epidemiologic projects at the clinical level, among isolated populations and on a broad international scale.

The most productive aspect of our research continues in Guam which is a treasure house of epidemiologic and genetic information. During the past year the significant observations reported in the publications by Hirano, et al., on the intracytoplasmic inclusion bodies and neurofibrillary changes associated with the exceedingly prevalent dementia-Parkinsonism syndrome and amyotrophic lateral sclerosis, has resulted in a new series of studies to evaluate a possible infectious etiology of these disorders on Guam and to study the interrelationship of chronic progressive neurological disorders of unknown cause, namely the "presenile" dementias, Parkinsonism and motor neurone disease. Opportunities for intensive research in diabetes, gout and diaphyseal aclasia are present among the Chamorros, but proceed slowly because of lack of a suitable research facility and personnel on Guam.

Because of the lack of laboratory space at NIH, it was necessary to assign Dr. Hirano to the Department of Neuropathology, Montefiore Hospital, New York, where he will conduct his comparative pathological study of Guam and "Stateside" Parkinsonism and amyotrophic lateral sclerosis. Dr. Simmons Lessell, Dr. Hirano's replacement on Guam, has already demonstrated his ability to conduct independent clinical research in convulsive disorders and the neurological complications of diabetes, as well as to procure needed necropsy specimens and clinical and genetic follow-up data for the numerous studies which the Branch conducts in Micronesia. The new Director of the Guam Department of Medical Services, appointed in mid-1960, has renewed the excellent relationship which our research group has had with the Guam administration.

On the local scene, Dr. Krooth has continued his laboratory investigations of the applications of tissue culture techniques to selected genetic diseases such as galactosemia. Tissue culture is a new and promising area and the observation of the behavior of cells from patients with genetic disease may have far-reaching implications in the elucidation of metabolic pathways and, ultimately, in the treatment of these diseases. Dr. Krooth has also collaborated with Dr. Hin Tjio, NIAMD, in investigating possible chromosomal abberations in human disease. No laboratory space is available for Dr. Krooth from NINDB; his work is being carried out in laboratory space loaned by NIAID.

Dr. Myrianthopoulos has continued his field and laboratory investigations on the genetics and epidemiology of neurologic disorders. The first phase of one study suggests that the frequency of Parkinson's disease is higher among the relatives of patients who develop extrapyramidal symptoms from ataraxic drugs than among the relatives of controls. In a study of conduction velocity time in peroneal muscular atrophy patients, there are peculiar electromyographic patterns which indicate that the lesion in this disease complex may be located either in the nerve, the roots, or the anterior horn cells in affected members of the same family. The collection of material for the blood group typing in amyotrophic lateral sclerosis patients nears completion, and an analysis will be made in 1961. A study of population genetics of Tay-Sachs disease is nearing completion, and a clinical and genetic study of congenital anomalies associated with myotonic dystrophy is continuing.

The study of the epidemiology of a neurological disorder in Minamata, Japan, mentioned in last year's annual report has been published. Investigations in Japanese and United States vinyl chloride plants have revealed a difference in processing which accounts for the intoxication by mercury compound of some of the population in Minamata through the ingestion of poisoned fish. The identification of the mercury toxin was first made in Japan; it was delayed for over a year at N.I.H. because of the lack of a suitable laboratory at NINDB and was finally carried out at the Sanitary Engineering Center (S.E.C.), PHS, Cincinnati, Ohio, when it could conveniently be fitted into the Center's schedule. Further studies are under way by the S.E.C. laboratory to ascertain the possible risk from pollution of the air by mercury in the distillation processes of vinyl chloride in the United States.

A project on the thallium poisoning epidemic which occurred in Southeastern Texas from 1955 to 1959 is still under way and an effort is being made to determine long-term sequelae of this known toxic agent.

The epidemiology of multiple sclerosis is still actively studied by this Branch; consultation in research methods is provided to foreign investigators in Japan, Iceland, Australia, New Zealand, Poland and other countries where such research is being pursued. Dr. Milton Alter who has completed a comparative study of multiple sclerosis in Charleston and Halifax and a study of the incidence of central nervous system malformations in Charleston, is currently investigating the prevalence of multiple sclerosis among ethnic groups in Israel. Dr. Fons is studying the incidence of multiple sclerosis in American Indians in the upper mid-West and in the Southwestern States. It was not feasible for him to complete his study of the cerebral vascular diseases in Rochester, Minnesota.

Dr. Richard Darrell, on assignment from the Communicable Disease Center, is aiding in the study of the incidence and geographic distribution of uveitis, which is being pursued through the records of the Mayo Clinic.

In addition, Dr. Darrell is planning a prospective survey of uveitis in other communities and a project on the epidemiology of congenital strabismus and of myopia among the school children of the Washington suburban area.

The Chief of the Branch participated in several of the aforementioned projects and has continued the study with the National Research Council and the Veterans Administration on the natural history of retrobulbar neuritis and multiple sclerosis.

The mounting emphasis in the Public Health Service on international research has led to an increased effort to clarify many questions in the geographic distribution of diseases of the nervous system. A world-wide mortality study of neurological diseases was initiated with the Biometrics Branch. There has been considerable activity in the newly-organized World Federation of Neurology (WFN). The Branch Chief is Chairman of the Biometry and Genetics Commission of WFN, has been active on program arrangements for the symposium on "The Contributions from Geographic Isolates to Epidemiology and Genetics in Neurology" at the forthcoming International Congress of Neurology; he is also concerned with the program of the forthcoming conference of the International Epidemiologic Association. It has been possible for the Branch Chief to integrate much of his international research activities and experience with his duties as the Institute's representative to NIH in connection with the Public Law 480 program and for the deliberations of the Task Force for Off-Site Installations.

It has been difficult to launch several new programs because of the lack of suitable space for essential supportive laboratory work at NIH and the disruption caused by the dispersion of personnel resulting from our unsatisfactory space assignments away from NIH. This Branch has been moved about or off the NIH reservation 9 times in less than 5 years; this practice places us under a tremendous handicap in fulfilling our obligations to the Institute, and has made almost impossible the recruitment of the scarce and highly specialized professional staff required for the Branch. This lack of a suitable space allotment has also jeopardized the final organization of the proposed Sections on Human Genetics, Environmental and Geographic Studies, and Animal Genetics and Epizotiology.

This Branch intends to carry out fully its unique responsibilities both within the NIH and in international research; prompt action on its critical space requirement will insure this in the coming fiscal year.

PUBLICATIONS

Published or in Press

1. Myrianthopoulos, N. C., and Rowley, P. T.: Monozygotic Twins Concordant for Huntington's Chorea. Neurology 10:506, 1960

2. Myrianthopoulos, N. C., and Mackay, R. P.: Multiple Sclerosis in Twins and Their Relatives: Genetic Analysis of Family Histories. Acta Gen. Stat. Med. 10:33, 1960

3. Myrianthopoulos, N. C., and Kurland, L. T.: Present Concepts of the Epidemiology and Genetics of Hydrocephalus. Houston Neurological Society Symposia, Charles Thomas, Publisher, in press.

4. Myrianthopoulos, N. C.: Review of "Human Heredity" by Ashley Montagu, Eugenics Quarterly, in press.

5. Krooth, R. S. and Weinberg, A. (1960): Properties of Galactosemic Cells in Culture. Biochem. Biophys. Res. Comm., in press.

6. Alter, Milton; Talbert, O. Rhett; Allison, R. S.; Kurland, Leonard T.: The Geographic Distribution of Multiple Sclerosis. A Comparative Study in Charleston County, South Carolina and Halifax County, Nova Scotia. I. Prevalence in Charleston County, South Carolina. Journal of the South Carolina Medical Association, 56:209, 1960

7. Alter, Milton; Talbert, O. Rhett; Allison, R. S.; Godden, John O.; Kurland, Leonard T.: The Geographic Distribution of Multiple Sclerosis. A Comparison of Prevalence in Charleston County, South Carolina and Halifax County, Nova Scotia. II. Prevalence in Halifax County, Nova Scotia. Nova Scotia Medical Bulletin, Nova Scotia Medical Bulletin, July, 1960.

8. Alter, Milton; Allison, R. S.; Talbert, O. Rhett; and Kurland, Leonard T.: Geographic Distribution of Multiple Sclerosis. World Neurology 1:55, 1960.

9. Okinaka, Shigeo; McAlpine, Douglas; Miyagawa, Kuheita; Suwa, Nozomi; Kuroiwa, Yoshigoro; Shiraki, Hirotsugu; Araki, Shukuro; and Kurland, Leonard T.: Multiple Sclerosis in Northern and Southern Japan. World Neurology 1:1, 1960.

Published or in Press - 2

10. Fullmer, Harold M.; Siedler, Howard D.; Krooth, Robert S.; and Kurland, Leonard T.: A Cutaneous Disorder of Connective Tissue in Amyotrophic Lateral Sclerosis. A Histochemical Study. Neurology 10:8, 1960

11. Alter, Milton; Talbert, O. Rhett; and Kurland, Leonard T.: Myasthenia Gravis in A Southern Community. AMA Archives of Neurology 3:399, 1960.

12. Alter, Milton and Talbert, O. Rhett: Myasthenia Gravis in One Monozygotic Twin. Neurology 10:793, 1960.

13. Kurland, L. T. and Alter, M.: The Epidemiology and Genetics of Myasthenia Gravis, Second International Congress on Myasthenia Gravis; in press, Charles Thomas, Publisher.

14. Hirano, Asao; Krooth, Robert S.; Kurland, Leonard T.: Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam. 1. Clinical Features, in press, Brain.

15. Hirano, Asao; Malamud, Nathan and Kurland, Leonard T.: Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam. II. Pathological Study, in press, Brain.

16. Kurland, Leonard T.; Faro, Stanley, and Siedler, Howard D.: Minamata Disease. The Outbreak of a Neurological Disorder In Minamata, Japan and its Relationship to the Ingestion of Seafood Contaminated by Mercury Compounds, World Neurology 1:5, 1960

17. Kurland, Leonard T.; Myrianthopoulos, N. C., Siedler, Howard D.: Epidemiology and Genetics. Report of an Extramural Survey and Program Committee, Neurological and Allied Sciences, Bardgett Printing and Publishing Co., St. Louis, Missouri, 1960.

*18. Fine, David I. M.; Barron, Kevin D.; and Hirano, Asao: Central Nervous System Lipidosis in an Adult with Atrophy of the Cerebellar Granular Layer. Journal of Neuropathology and Experimental Neurology 19:3, 1960.

*19. Friedman, Arnold P.; Carton, Charles A.; Hirano, Asao: Facial Pain. Postgraduate Medicine 27:6, 1960.

Published or in Press - 3

*20. Friedman, A. P.; Carton, Charles A.; and Hirano, Asao: Cyclical Head and Face Pain. AMA Archives of Neurology 2:1, 1960

*21. Barron, Kevin D.; Siqueira, Edir; Hirano, Asao: Cerebral Embolism Caused by Nonbacterial Thrombotic Endocarditis. Neurology 10:4, 1960.

*Papers 18 thru 21 were initiated by Dr. Hirano prior to his joining this Branch.

REPORTS (Unpublished data)

I. Myrianthopoulos, N. C., Kurland, L. T., and Kurland, A.: A Genetic Study of Parkinsonism Produced by Ataraxic Drugs.

II. Menkes, John H., and Alter, Milton: A New, Possibly Sex-Linked Recessive Disorder with Growth Retardation, Puculiar Hair (Phili Torti) and Severe Mental and motor impairment. Paper presented at the American Academy of Neurology, April 1960.

ELECTED TO MEMBERSHIP

International Epidemiological Association, May, 1960. Dr. Kurland

1960 - Clinical Professor of Neurology, Howard University, Dr. Kurland

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Clinical Study of Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam

Principal Investigator: Asao Hirano

Other Investigators: Robert S. Krooth
Leonard T. Kurland

Man-Years: Total - 1-1/2
Professional - 1/2
Other - 1

Project Description:

This is a clinical study of a neurological disorder manifested by Parkinsonian syndrome with mental deterioration found among the indigenous Chamorros of Guam. It affects Chamorro adults in the fourth to sixth decades, and death usually results about three to five years after the recognized onset. The onset is insidious and the main clinical features are mental deterioration of the organic type (dementia) and the Parkinsonian syndrome. In addition, signs of upper motor neurone involvement or the ALS syndrome are observed in about 1/3 of the cases. Many patients have a family history of ALS, "Parkinsonism", or both.

Although the disease may demonstrate the cardinal features of mental deterioration, extrapyramidal disease and motor neurone disease of the Jakob-Creutzfeldt disease, it cannot at present be considered as analagous to any one of the numerous clinical variants included in this grouping. There are also indications that this disorder on Guam is not of a postencephalitic nature. Pathological and other studies now under way are expected to clarify these matters.

PART B.

Paper to be published in Brain, 1961.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Pathological Study of Parkinsonism-Dementia Complex, An Endemic Disease on the Island of Guam

Principal Investigators: Asao Hirano
Leonard T. Kurland

Other Investigator: Nathan Malamud, Professor of Psychiatry and Neuropathology, University of California, San Francisco, California.

Man-Years: Total - 1-1/2
Professional - 1/2
Other - 1

Project Description:

This is a pathologic study of seventeen cases of a neurological disorder manifested by a Parkinsonian syndrome with mental deterioration found among the Chamorros of Guam. The gross neuropathological features are the presence of cortical atrophy, and depigmentation of the substantia nigra and locus caeruleus. The consistent finding on microscopic examination is wide-spread ganglion cell degeneration of the central nervous system. It affects primarily the cortex of the frontal and temporal lobes, Ammon's horn, amygdaloid nucleus, hypothalamus, globus pallidus, thalamus, periaqueductal structures, substantia nigra and tegmentum of the brain stem. There are numerous intra-ganglionic fibrillary changes and scattered intracytoplasmic granulovacuolar bodies in the affected neurones of these areas. However, typical senile plaques are not found in any of the cases. In addition, there is a diffuse loss of ganglion cells of a variable degree, most marked in the globus pallidus and substantia nigra. Reactive glial proliferation accompanies the neuronal involvement. Myelin sheaths are usually spared with the exception of the cases which presented clinically with features of amyotrophic lateral sclerosis and pathologically with degeneration of motor neurones, as well as bilateral demyelination of the pyramidal tracts. Further studies on the relationship of this disorder to ALS and efforts to determine the course of this disorder continue.

PART B.

Manuscript to be published in Brain.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Investigations of Chamorros on the Island of Guam

Principal Investigators: Leonard T. Kurland
Asao Hirano
Simmons Lessell
Jose Torres
Nathan Malamud
Leon Rosen

Other Investigators: Joseph Kovacs, Director
Department of Medical Services
Territory of Guam

Man-Years: Total - 4
Professional - 2
Other - 2

Project Description:

The stable population, small size of the Island, high quality of available medical care, large sibships and high incidence of certain chronic diseases amongst the native population make Guam a valuable natural epidemiological laboratory. Investigations of amyotrophic lateral sclerosis are still in progress. In addition, efforts are underway to define the characteristics of the presenile-dementia-extrapyramidal syndrome discovered to be so prevalent on Guam. Simple goiter appears to be unduly frequent on the Island, a finding which gains even greater significance in light of the high iodine content of the native diet. Diabetes mellitus appears to be excessively prevalent amongst the Chamorros and a survey of neurological illness in this subgroup has been completed. Hyperurecemia is also very frequent amongst the Chamorros. Finally, seizure disorders on Guam have come under the scrutiny of this Branch because of their alleged frequency and because epidemiological data regarding seizure disorders are not presently available for tropical areas.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Seizure Disorders

Principal Investigators: Simmons Lessell
Leonard T. Kurland

Other Investigator: Jose Torres

Man-Years: Total
Professional
Other

Project Description:

Physicians working on Guam have been aware for some time of an apparently high incidence of febrile seizures amongst the Chamorros children. A survey was undertaken in a typical Guamanian village using the public grammar school children as index cases. The prevalence of positive seizure histories in the children and their siblings was found to be 9.6/100. Only four of the 78 individuals with positive seizure histories had ever experienced an afebrile seizure. A significantly higher prevalence of positive histories was obtained from the parents and siblings of "affected" index cases than from the parents and siblings of "non-affected" individuals. Further studies are under way.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Neurological Disease in Diabetes Mellitus Patients

Principal Investigators: Simmons Lessell
Leonard T. Kurland

Other Investigators: Jose Torres
Joseph Kovacs, Director
Department of Medical Services
Territory of Guam

Project Description:

A survey of Chamorro adults completed in the latter part of 1959 revealed a prevalence of 12.6/100 for diabetes. A random segment of this diabetic population was selected for a study to determine the incidence of neurological disease amongst Chamorro diabetics. One hundred such individuals were subjected to a neurological interview and examination. Five cases of the Presenile-Dementia Extrapyramidal Syndrome were encountered. Five cases of cerebral vascular disease were encountered in the group and 8 instances of diabetic peripheral neuropathy were noted. Three patients had suffered a Bell's Palsy. Further work on the relationship of diabetes to the neurological disorders is contemplated.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Serological Survey Among Guamanians

Principal Investigator: Simmons Lessell

Other Investigator: Leonard T. Kurland

Collaborating Investigators: (To be designated)

Project Description:

The histopathologic findings of neurofibrillary tangles and intracytoplasmic granulovacuolar bodies in the central nervous system of patients dying with the dementia-Parkinsonism and ALS syndrome on Guam again raises the question of a viral etiology.

Dr. Lessell is collecting sera from patients and the unaffected population. At a laboratory to be selected titers will be measured for antibodies against arbor and other viruses, especially those occurring predominantly in tropical areas.

This survey will be extended to include the Chamorro population on Rota and Saipan and the Carolinians of Saipan and one of the Caroline Islands.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Relation of Ability to Taste Phenylthiourea to Thyroid Disease

Principal Investigator: Robert S. Krooth

Other Investigators: Dr. T. E. Ross, NIAMD
Dr. Bernard Witkopf, NIAMD

Man-Years: Total - 3/12
Professional - 3/12

Project Description:

As noted, in detail in last year's report, the association of certain types of thyroid disease with ability to taste phenylthiourea (and related compounds) has been observed by several groups of investigators. Evidence bearing on this association is being gradually accumulated, but our series is too small at present to permit a meaningful statistical analysis.

The studies, carried on in collaboration with Dr. Witkopf, on the chemical nucleus conferring taste activity have thus far led to no clear conclusions. The two difficulties have been the low solubility of the compounds in usual organic solvents, and the difficulty in determining how much activity a particular compound has.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Karyotypic Abnormalities in Human Disease

Principal Investigator: Hin Tjio, NIAMD

Collaborating Investigator: Robert S. Krooth

Man-Years: Total - 3/12
Professional - 3/12

Project Description:

A search is being made in our laboratory, as in others, for new chromosomal abnormalities in human disease. Cell lines have been developed from biopsies on 5 patients with congenital malformations, in each case of a kind not previously reported. In every instance thus far the chromosomes have been normal in morphology and number.

Certain other studies on the human chromosomes are being carried out.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Application of the Technique of Tissue Culture to Selected Genetics Diseases

Principal Investigator: Robert S. Krooth

Collaborating Investigators: Dr. Arnold W. Weinberg, NIAMD
Dr. Paul Altrocci, NINDB
Dr. Harry S. Eagle, NIAID

Other Investigators: Dr. Herman S. Kalckar, Department of Biochemistry
Johns Hopkins University
Dr. Howard B. Hamilton, U.S. Atomic Bomb
Casualty Commission, Hiroshima, Japan

Man-Years: Total - 6/12
Professional - 6/12

Project Description:

Studies to follow a number of genetic diseases in tissue culture are being continued. Biopsies are performed on affected patients, and cell lines are then developed from the excised tissue. Evidence bearing on the persistence of the defect in cell lines from affected persons (compared with cell lines from controls) is obtained. Ultimately, mutant cell lines from patients with inborn metabolic errors should prove useful in the study of genetic exchange among human cells as well as in other aspects. The system permits one to study mutant human cells in a way which hitherto could be applied only to mutants of bacteria and fungi. However, the number of genetic diseases which are susceptible to this approach is at present quite limited.

Galactosemia has been shown to persist as defect in culture, even after 6 months of propagation and an increase in cell number known to exceed 30-billion fold. The defect can be shown by growth studies, the ability of the cells to metabolize galactose-1-C^{14}, and direct enzymatic assays. The galactosemic cells show a pattern of galactose

sensitivity similar to the transferaseless mutants of E. coli. In each case the galactosemic cells are compared with control ones. Corresponding data have been obtained for one heterozygote, and here also the defect persists.

Wilson's disease and hypocatalasemia are also under study by this approach.

Our efforts to follow Gaucher's disease and certain other histiocytic states in culture (referred to in the last report) have not yet succeeded. Indefinite propagation of recognizeably affected cells has not been achieved although the cells will attach to glass, spread, and appear (at least) to increase in number for a few weeks. Thereafter, though the cells can be made to survive for months, no evidence of growth is found.

PART B.

Krooth, R. S., and Weinberg, A. (1960) Properties of Galactosemic Cells in Culture. Biochem. Biophys. Research. Comm. (in press)

PHS - NIH
Individual Project Report
Calendar Year - 1960

PART A.

Project Title: The Outbreak of a Neurological Disorder in Minamata, Japan and Its Relationship to the Ingestion of seafood Contaminated by Mercury Compounds

Principal Investigator: Leonard T. Kurland

Other Investigators: Stanley N. Faro, Howard D. Seidler

Collaborating Institutions: Kumamoto University School of Medicine Tokyo University, Department of Neuropathology, Sanitary Engineering Center, PHS, Cincinnati, Ohio

Project Description:

An epidemic of a chronic progressive and often fatal neurological disorder occurring in families residing in fishing villages near Minamata Bay has been under study by Japanese scientists since 1953. Pathological examination of central nervous system specimens suggested a similarity to organic mercury poisoning. Mercury in high concentration was found in marine life from Minamata Bay and from mud especially near the effluent channel of a large plant manufacturing vinyl chloride. Mercuric chloride catalyst is lost in appreciable quantities from this plant. Clinical and laboratory studies here have confirmed these findings. Studies in the vicinity of U. S. vinyl chloride plants revealed that a difference in the purification process prevented undue contamination of nearby waters although air pollution with mercury had to be guarded against. Recommendations have been made to reduce the threat of continued outbreaks in the vicinity of the Japanese plants.

PART B.

Kurland, Leonard T.; Faro, Stanley, and Siedler, Howard D. Minamata Disease. The Outbreak of a Neurological Disorder in Minamata, Japan and its Relationship to the Ingestion of Seafood Contaminated by Mercury Compounds, World Neurology 1:5, 1960

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Epidemiologic Investigation of Outbreak of Thallium Poisoning in Southeastern Texas, 1955-59

Principal Investigator: Stanley N. Faro

Other Investigators: Anthony L. Fons
Leonard T. Kurland
James W. Crawley
Samuel J. L. Pieper

Project Description:

An outbreak of Thallotoxicosis occurred in Southeastern Texas from January 1955 through December 1959; 107 children through age 14 were hospitalized during this 4-year period for accidental ingestion of thallium sulfate-containing rodenticides and insecticides. Sixty of these are known to have ingested one particular roach poison (Echols). There is reason to believe that many more children were seen in outpatient clinics and private offices and were not hospitalized.

Roaches and ants are prevalent in the humid gulf coast region of Texas and numerous compounds have been manufactured by exterminators in an effort to rid the area of these pests. In the late '40's exterminators in Kingsville, Texas were using a mixture of thallium sulfate, cereal and syrup as a rodenticide. Two deaths were reported in 1948 among 4 children who ate this mixture as "candy". In 1952 The Echols Mfg. Co. of Corpus Christie bought the business of one of the Kingsville Exterminating Companies and began the manufacture of a rodenticide containing 3% thallium sulfate in a palatable vanilla wafer base.

During the early part of 1957 the legislature of the State of Texas enacted legislation, enforced by the Division of Food and Drugs of the State Department of Health in Austin, Texas, restricting the manufacture of any mixture containing thallium sulfate or any thallium salt to contain not more than 1% of thallium. The study includes a

review of clinical and pathological features, biochemical effects, and long-term follow up for neurological and other sequelae.

PART B.

A report is being prepared.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Epidemiologic Investigation of Fish Poisoning in the South Pacific

Principal Investigator: Albert H. Banner, Director
Hawaii Marine Laboratory; Philip Helfrisch, Assistant Marine Biologist, Hawaii Marine Lab.

Co-Investigator: Leonard T. Kurland

Man-Years: Total - 1
Professional - 1/2
Other - 1/2

Project Description:

Poisoning from the ingestion of fish occurs in many areas of the world. Little is known of the specific toxic agent or the reasons for its sporadic and often unpredictable development. There has apparently occurred an increase in the incidence of fish poisoning with its variety of neurological symptoms in the Western and South Pacific area. It is of sufficient magnitude to warrant further investigation to delineate more exactly the nature of fish poisoning and to obtain an estimate of the morbidity and mortality following ingestion of such fish.

To facilitate the epidemiologic study, the Branch is assisting the above-named investigators in the preparation of the study design, the drafting of a pamphlet to guide medical practitioners and physicians in Oceania participating in the study and the drafting of a questionnaire designed to ascertain the fundamental epidemiologic characteristics of fish poisoning. As necessary and where possible, when severe outbreaks of fish poisoning occur, competent medical staff, including neurologists when possible, and biologists will visit the scene to investigate the outbreak and to aid the local authorities.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Does Multiple Sclerosis Exist Among American Indians?

Principal Investigator: Anthony Fons

Collaborating Investigator: Leonard T. Kurland

Man-Years: Total - 1/8
Professional - 1/8

Project Description:

There has been no published material containing epidemiological data on the frequency and distribution of multiple sclerosis or other demyelinating diseases among the American Indians. The Indian population resides in areas that vary widely in climate, geography and natural resources.

The relatively recent introduction of a cross-indexing system in the PHS medical care installations has greatly facilitated the review of records for specific diagnoses. The record systems of three Indian Reservations in North and South Dakota were reviewed recently in an attempt to establish the absence or presence of possible cases of multiple sclerosis.

Because of this opportunity to remedy the paucity of information on M.S. in this ethnic group living in varied socio-economic, geographic, and environmental conditions with high morbidity and mortality rates from infectious disease, exploration of an extension of the "pilot" three-Reservation survey to include other Indian populations is currently being undertaken.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The incidence of Anencephalus, Hydrocephalus and Spina Bifida in Charleston County, S. C., 1946-1955

Principal Investigator: Milton Alter

Man-Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

Anencephalus, hydrocephalus, and spina bifida represent the major types of congenital malformations of the central nervous system. A survey of hospital mortality, clinic and physicians' records in Charleston County, South Carolina for the period 1946-1955 disclosed 140 cases with these malformations

The incidence of anencephalus, hydrocephalus, and spina bifida per 1000 live births was 0.53, 0.96, and 0.71 respectively. Anencephalus and spina bifida is less common in negroes than whites, but hydrocephalus is equally frequent in the two races. Anencephalus and spina bifida are more common in females. Hydrocephalus is more common in males. The frequency of anencephalus is correlated with maternal age. Familial aggregation of cases was not observed except for an instance of anencephalus in twin negro girls. No seasonal or secular trend in incidence was noted.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Prevalence of Multiple Sclerosis Among Ethnic Groups in Israel

Principal Investigator: Milton Alter

Other Investigators: L. Halpern, Hadassah University Hospital
J. Silberstein, Israeli Ministry of Health
L. Bornstein, Israeli Ministry of Health

Cooperating Units: Hadassah University Hospitals
Israeli Ministry of Health
Kuppat Cholim Medical Organization

Man-Years: Total - 2
Professional - 1
Other - 1

Project Description:

The highly diverse population of Israel, consisting of elements drawn from many parts of the world offers a unique opportunity to determine the relative prevalence of Multiple Sclerosis in various ethnic groups. Intensive efforts are being made to locate and examine diagnosed cases of Multiple Sclerosis and clinically similar disorders.

Clinical impressions among neurologists working in the country many years, indicate that relatively few cases occur among Jewish immigrants from Middle Eastern countries and native-born Jews while ethnic groups from Northern and Western Europe are relatively frequently affected.

Preliminary results tend to confirm this impression with a single exception, i.e., Jews born in Iran (Persia) seem to have an unusually high prevalence of Multiple Sclerosis, although recent information suggests that the Jews in Israel from Persia had been born in Eastern Europe and had migrated to Persia in the early part of the century. If the clinical impression regarding the relative prevalence of Multiple Sclerosis among ethnic groups can be confirmed, studies of dietary differences, life patterns, genetic factors, immunological responses and other appropriate investigations will be initiated among samples of the affected individuals and suitable controls in an effort to account for the prevalence difference.

PART B.
None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Multiple Sclerosis Among American Negroes

Principal Investigator: Milton Alter

Cooperating Units: Neurological Institute, New York

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

There is some evidence that hereditary factors may influence the occurrence of Multiple Sclerosis. It follows that certain races may show a relative resistance to this disease or that the disease may assume atypical forms in certain races.

There is little information on the occurrence of Multiple Sclerosis among Negroes. If this race were relatively resistant to the disease or manifested atypical forms, the relatively low prevalence of Multiple Sclerosis in subtropical and tropical areas, largely inhabited by negroes, might be explained.

In an effort to accumulate information on manifestation of M.S. in the Negro race, the records of cases seen at the Neurological Institute of New York between 1947 and 1957 were reviewed. Approximately 100 cases in negroes were found and are being analyzed for clinical characteristics such as age and mode of onset, course, systems affected.

Correlation between the clinical features and length of residence in the North are being made.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Cerebellar Ataxia, Congenital Cataracts, and Retarded Physical and Mental Maturation in a Negro Family. (Marinesco-Sjogren Syndrome)

Principal Investigator: Milton Alter

Man-Years: 1/10

Project Description:

A rare hereditary syndrome characterized by cerebellar ataxia, congenital cataracts and retarded physical and mental maturation was first described by Marinesco in 1937. Since then several additional families have been found and the mode of inheritance characterized as autosomal recessive.

The first family so affected to be identified in America and the first in Negroes is currently being studied. The mode of inheritance may be autosomal dominant in this family.

Biochemical studies on two of the affected individuals are being carried out in an effort to identify an "inborn metabolic error".

PART B.

None

The relation between such factors as maternal age, parity, maternal health, fertility, place of residence, social status and the frequency of congenital malformations of the nervous system were reviewed. Problems in classification of malformations were mentioned.

Possible etiologic factors including heredity, infection, undernutrition, intoxication, climate, allergic reactions were examined in the light of current epidemiologic data.

PART B.

None

PHA - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Epidemiology of Congenital Malformations of the Nervous System with Special Reference to a Survey in Charleston, South Carolina

Principal Investigator: Milton Alter

Man-Years: Total - 2/12

Project Description:

Aspects of the frequency and population selectivity of anencephalus, hydrocephalus and spina bifida in Charleston County, South Carolina, were studied for the period 1946 through 1955.

An incidence of between 2 and 3 per 1000 live births was found for the combination of these malformations. Hydrocephalus was most common (0.94 per 1000 live births); spina bifida was second in frequency (0.69 per 1000 live births); and anencephalus was least common (0.53 per 1000 live births). The association of hydrocephalus and spina bifida was shown not to be fortuitous. The incidence of spina bifida and anencephalus was only half as great in negro infants as in whites, but hydrocephalus occurred with equal frequency in the two races.

A large female preponderance was found in anencephalic infants in both negro and white races. Spina bifida cases showed only a slight female preponderance, whereas among hydrocephalic infants, males predominated.

The data from the Charleston survey were compared with similar data gathered from various parts of the world. Charleston, with respect to the range of frequencies of hydrocephalus and spina bifida, falls rather in the middle but has a relatively low incidence of anencephalus. No secular or seasonal pattern of incidence was discernable in the Charleston data in contrast to reports of a falling incidence of anencephalus and spina bifida reported from other areas and an alleged tendency for anencephalic births to occur in the colder months.

The relation between such factors as maternal age, parity, maternal health, fertility, place of residence, social status and the frequency of congenital malformations of the nervous system were reviewed. Problems in classification of malformations were mentioned.

Possible etiologic factors including heredity, infection, undernutrition, intoxication, climate, allergic reactions were examined in the light of current epidemiologic data.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Prospective Study of the Epidemiology and Geographic Distribution of Uveitis in Three Eastern Communities.

Principal Investigator: Richard W. Darrell

Other Investigator: Leonard T. Kurland

Collaborating Institute: (To be designated)

Man-Years: Total - 1/2
Professional - 1/4
Other - 1/4

Project Description:

The new cases of uveitis occurring during 1961 in the resident populations of three Eastern cities of about 50,000 will be analyzed with respect to characteristics of the patient, of the environment, and of the natural history of the disease.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Retrospective Survey of the Incidence and Geographic Distribution of Uveitis in Rochester, Minnesota

Principal Investigator: Henry P. Wagener, Mayo Clinic, Rochester, Minnesota

Other Investigators: Leonard T. Kurland
Richard W. Darrell

Man-Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

There have been no total population surveys on the incidence, prevalence and distribution of uveitis. These parameters are now being studied through a retrospective study of the Mayo Clinic records of the resident population of Rochester, Minnesota during a 10-year period. Approximately one-half of the records have been abstracted.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Prospective Study of Epidemiology and Geographic Distribution of Congenital Strabismus in Three Eastern Communities

Principal Investigator: Richard W. Darrell

Other Investigator: Leonard T. Kurland

Collaborating Investigator: (To be designated)

Man-Years: Total - 1/2
Professional - 1/4
Other - 1/4

Project Description:

In conjunction with a prospective study of uveitis, new cases of congenital strabismus occurring during 1961 in the resident populations of three Eastern cities will be analyzed epidemiologically.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Association of Blood Groups to Amyotrophic Lateral Sclerosis

Principal Investigator: N. Myrianthopoulos

Other Investigators: P. Schmidt, LBBP
Leonard T. Kurland

Cooperating Units: Laboratory of Blood and Blood Products
Division of Biologic Standards

Man Years: Total - 2/12
Professional - 2/12

Project Description:

A pilot study to determine if there exists any selection for a specific blood type among patients with motor neurone disease.

Present Status: The medical facilities of the Army, Navy, Air Force and Veterans Administration, the Guam Research Center, and many practicing neurologists have been providing blood and saliva specimens as in the past. Collection of specimens will continue for approximately six more months at which time an adequate sample for analysis is expected to have been accumulated.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Genetic and Clinical Investigations in Myotonic Dystrophy

Principal Investigator: N. Myrianthopoulos

Other Investigators: J. Caughey, NINDB (Now at the University of Otago, Dunedin, N.Z.)

Man-Years: Total - 3/12
Professional - 1/12
Other - 2/12

Project Description:

A study based on history and examination to determine if children of patients with myotonic dystrophy show congenital anomalies more frequently than expected; if there exists a significant difference in the occurrence of congenital anomalies between children of parents with the complete syndrome and children of patients who show only cataract; if the sex of the affected parent has any effect in the production of congenital anomalies among the children; and what is the reproductive fitness of persons affected with myotonic dystrophy.

Present Status: Extensive and detailed pedigrees have been obtained for five families in which myotonic dystrophy has occurred. It is felt that at least five more families must be thoroughly worked out before an analysis can be attempted. Since these families are scattered throughout the U.S. and extensive field work is required to obtain the necessary clinical and genetic information, the investigation is practically restricted to the summer months when COSTEP help for field work is available.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Detection of the Genetic Carrier in Peroneal Muscular Atrophy by Conduction Velocity Studies

Principal Investigator: N. Myrianthopoulos

Other Investigators: M. Lane, NINDB
D. Silberberg, NINDB

Man-Years: Total - 3/12
Professional - 1/12
Other - 2/12

Project Description:

A study to determine whether the genetic carrier (whether unaffected children of affected parents or individuals showing lack of penetrance) can be detected by slow peripheral nerve conduction velocity. A slowing of conduction velocity in the peroneal, ulnar and other nerves has been repeatedly demonstrated in patients with peroneal muscular atrophy.

Present Status: About 100 affected, suspected genetic carriers and normal members of four families with peroneal muscular atrophy have been tested for conduction velocity of their anterior tibial and ulnar nerves. The results have not yet been evaluated. In the course of these studies some findings suggested that a distinction may perhaps be drawn between a purely peripheral and a myelopathic form of the disease on the basis of electromyographic findings. It was, therefore, decided to extend the investigations to more individuals in order to confirm this impression.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Detection of the Heterozygote in Cerebroretinal Degeneration (Amaurotic Family Idiocy, Juvenile or Spielmeyer-Vogt Type)

Principal Investigator: N. Myrianthopoulos

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

Examination of a large number of peripheral blood smears from patients with the infantile form of cerebroretinal degeneration, and smears from their parents and siblings, failed to disclose any diagnostic changes in the peripheral blood. Examination of blood smears from one patient with the Juvenile form of the disease disclosed very striking changes in the lymphocytes in the form of large vacuoles in the cytoplasm. Blood smears from eight other patients with the juvenile form, their parents and siblings did not show these changes.

ll reports in the literature of vacuolated lymphocytes in this isease come from the Scandinavian countries. Several investigators n the U.S. reported that they failed to observe them in their atients. It is interesting to note that the family of the patient ho showed vacuolated lymphocytes emigrated to the U.S. from Sweden. t is possible that we are dealing with a Scandinavian variant of his disease. More cases are now being sought to confirm this ypothesis.

PART B.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Some Epidemiologic Features of Tay-Sachs Disease

Principal Investigator: N. Myrianthopoulos

Man-Years: Total - 3/12
Professional - 2/12
Other - 1/12

Project Description:

A study to determine the incidence and prevalence of Tay-Sachs disease among the Jewish and non-Jewish population of the United States by mortality statistics.

Present Status: Mortality statistics for the years 1954, 1955, 1956 and 1957 have been collected. Information concerning the family histories of the deceased and pertaining to the ethnic origin, religious affiliation and consanguinity of the parents, and the occurrence of the disease among the siblings has been obtained. The data are now being analyzed and a report is being prepared.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Multiple Congential Anomalies in Three Children of a Family

Principal Investigator: N. Myrianthopoulos

Other Investigators: R. Hamburger
C. Cheney

Cooperating Units: Yale University School of Medicine
Grace-New Haven Hospital

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

A genetic, clinical, anatomical and pathological study of what appears to be a new syndrome not previously described and characterized by multiple congenital abnormalities. These have appeared in three successive children of a New England family, all of whom died within one month after birth. A report is being prepared.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Parkinsonism - Ataraxic Drugs Study

Principal Investigator: N. Myrianthopoulos

Other Investigators: Leonard T. Kurland
A. Kurland, Spring Grove State Hospital

Cooperating Unit: Spring Grove State Hospital

Man-Years: Total - 6/12
Professional - 3/12
Other - 3/12

Project Description:

A study to determine the occurrence of Parkinson's disease among the relatives of two groups of patients: Those who show Parkinsonian symptoms on high therepeutic dosages of certain phenothiazine derivatives, and those who prove to be resistant to the side effects of these drugs. The two groups of patients, actually patients and controls, have been selected from the patient population of Spring Grove State Hospital.

Present Status: The study is now completed. Among 728 relatives of 59 patients susceptible to ataraxic drugs, 13 cases of Parkinson's disease were found. Of these, one is a bona fide post-encephaletic case. Among 777 relatives of 67 controls resistant to ataraxic drugs, three cases of Parkinson's disease were found. All three were described by the attending physicians as being of the arteriosclerotic type. Secondary cases of Parkinson's disease were found in two cases (13%), one among the relatives of the patient group and one among the relatives of the control group.

These findings were analysed by breaking them down by age and comparing them to age-adjusted, expected prevalence rates from Kurland's data. In all age groups the observed rate was higher than the expected in the relatives of the patient group by at least a factor of 4 which is significant. It was not different

in the relatives of the patient group. No further interpretation is attempted at this time. The data are being implemented by a similar study at St. Elizabeth's Hospital

PART B.

Myrianthopoulos, N. C., Kurland, L. T. and Kurland, A.: A Genetic Study of Parkinsonism Produced by Ataraxic Drugs. Report presented at a symposium on Parkinson's Disease, Arden House, N. Y., March 1960. Publication plans uncertain.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Survey of Schizophrenics Among the Relatives of Schizophrenia Patients

Principal Investigator: N. Myrianthopoulos

Cooperating Unit: Spring Grove State Hospital
St. Elizabeth's Hospital

Man-Years: Total - 1/12
Professional - 0.5/12
Other - 0.5/12

Project Description:

A study to determine the occurrence of schizophrenia among the relatives of patients who have already been selected for another project (Parkinsonism - ataraxic drugs) and to determine the role of inheritance in Schizophrenia. This project being a by-product of another project, has the advantage of overcoming some of the biases involved in selecting an adequate sample. The collection of data from Spring Grove State Hospital has been completed. These will be implemented with data from St. Elizabeth's Hospital concurrently with the project on ataraxic drugs conducted there before genetic analysis is attempted.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: A Genetic Study of Parkinsonism and the Dystonias Produced by Ataraxic Drugs

Principal Investigator: N. Myrianthopoulos

Other Investigators: Francis Waldrop

Cooperating Unit: St. Elizabeth's Hospital

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

A study to replicate the conditions and confirm the findings of the Parkinsonism-Ataraxic Drugs study. If the impression is confirmed that there is an hereditary susceptibility to Parkinsonism produced by phenothiazine derivatives then an experimental procedure will be followed to test a genetic hypothesis: Two groups of patients will be selected from St. Elizabeth's Hospital, one susceptible and one resistant to ataraxic drugs. The basis for selection of both groups of patients would be that they have a living relative with a coefficient of relationship of 0.5 (percent of sibling) who is now a patient at St. Elizabeth's Hospital and available for treatment with ataraxic drugs. A selected drug would then be administered to these relatives and their reactions observed and graded. The same procedure will be used with relatives of patients who show severe dystonia when given ataraxic drugs. The known degree of relationship of relative to patient will serve as a basis for prediction of the eventual outcome if susceptibility to ataraxic drugs is inherited along Mendelian lines.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: Epidemiology and Genetics of Hydrocephalus

Principal Investigator: N. Myrianthopoulos

Other Investigators: L. T. Kurland

Man-Years: Total - 1/12
Professional - 1/12

Project Description:

A synthesis and interpretation of the available data on the subject. The work of various investigators regarding the etiology, pathogenesis, geographic distribution, population and sex selectivity, relation to other CNS defects and genetic background of hydrocephalus has been critically reviewed and certain interpretations and recommendations made. They were presented at the annual meeting of the Houston Neurological Society, Houston, Texas, in March, 1960.

PART B.

Myrianthopoulos, N. C., and Kurland, L. T.,

Present Concepts of the Epidemiology and Genetics of Hydrocephalus. Houston Neurological Society Symposia, Charles Thomas, Publisher, in press.

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: International Mortality Study

Principal Investigator: Irving Goldberg

Other Investigator: Leonard T. Kurland

Man-Years: Total - 3/12
Professional - 3/12

Project Description:

This is a study of worldwide mortality from more than 25 neurological or related disorders. Material suitable for analysis for age adjusted rates for some or all of the diseases of interest and presently available for all 30 countries. Data were sought from those countries from which it was believed the desired statistics could be made available. Additional countries were contacted because of their geographic locations and ethnic compositions even though it was thought unlikely that the requested information could be provided in adequate detail. It is anticipated that an analysis of the material will be completed in time for presentation at the World Federation of Neurology meeting in September, 1961, because of its international scope.

PART B.

None

PHS - NIH
Individual Project Report
Calendar Year 1960

PART A.

Project Title: The Effect of Climate and Other Environmental Factors in the Progress of Multiple Sclerosis. One Aspect of a Broader Investigation of the Natural History of Multiple Sclerosis

Principal Investigator: Leonard T. Kurland

Other Investigators: Gilbert Beebe and Dean Nefzger, Follow-up Agency, National Research Council, Washington, D. C.
J. F. Kurtzke, Chief, Neurology Service, V. A. Hospital, Coatesville, Pennsylvania
Thomas Auth, Neurology Dept., Veterans' Administration, Washington, D. C.

Cooperating Units: Follow-up Agency, NRC, Washington, D. C.
Veterans' Administration, Washington, D. C.

Man-Years: Total - 3/12
Professional - 2/12
Other - 1/12

Project Description:

Patients diagnosed as having had retrobulbar neuritis, 1942-1945, are now being examined by neurologists. Preliminary results indicate a low frequency (about 10%) Multiple Sclerosis, as contrasted to 45-50% expected on the basis of available data in medical literature.

PART B.

None

Annual Report
Calendar Year 1960
Laboratory of Perinatal Physiology
Collaborative and Field Research
National Institute of Neurological Diseases and Blindness

William F. Windle, Acting Chief

As the fourth year of operations began at the Field Station in Puerto Rico, a reorganization and expansion program was undertaken. The project now is a Laboratory, administered as a direct operation of the Office of Director, NINDB, by the Assistant Director.

Initially conceived as a collaborative neurological study, involving scientific personnel of the Laboratory of Neuroanatomical Sciences, NINDB, and the Medical School of the University of Puerto Rico, to investigate the role of certain factors in the rhesus monkey's prenatal life and birth which lead to defects in the offspring, the investigations of the first three years made clear the desirability of broadening its scope and extending it to disciplines other than neurology. The rhesus monkey has proven to be an exceptionally valuable animal for several types of laboratory research in perinatal physiology. Relatively little previous experimental work had been done with timed pregnancies in this species.

Plans for the Laboratory are in process of formulation. One basic principle will be to provide facilities and resources both for a full-time scientific staff, and a changing group of visiting scientists of kindred interests from other institutions. To obtain maximum utilization of the material available, it is proposed to incorporate facilities for biochemistry, physiology (general and specialized), morphology, (including fine structure and histochemistry), psychology and behavior, ecology (including certain experimental aspects), pathology, and physical biology. Studies will not be confined to the nervous system, but will include the blood, cardiovascular, respiratory, endocrine, and reproductory systems; nutritional and other factors will be considered. Although the monkey (Macaca mulatta) will continue to be the primary laboratory animal used for these studies, other species of primates, and lower animals, will be required. The Laboratory in Puerto Rico will offer unique opportunities to combine research on confined monkeys with studies of monkeys in a natural habitat. The free-ranging colony at

Cayo Santiago will be supplemented by other island colonies. Furthermore, facilities for limited free-ranging will be developed on the islands for certain types of experiments that have been planned.

The scientific personnel has changed from time to time during the last 4 years, the longest tour of duty for any individual being approximately 2 years. With the establishment of the Laboratory on a permanent basis, it is expected that scientists will wish to remain for longer periods, and recruitment should be facilitated considerably.

Progress during the past year is reported under two headings: laboratory investigations centered in San Juan at the School of Medicine and the U. S. Public Health Service Out-Patient Clinic and Quarantine Station; and field studies centered on Cayo Santiago. The over-all accomplishments of the first four years may be sensed in part by reference to a list of publications, represented by Exhibit "A".

Investigations in the Laboratory. The laboratory colony of Macaca mulatta at Bethesda is in process of consolidation with that in San Juan. There will be about 225 females of breeding age in the combined colony. Additional temporary quarters for them are under construction on the USPHS grounds.

Efforts to establish values for menstrual and ovulatory cycles of these caged females have continued. As in man, the monkey menstrual cycle fluctuates from period to period, and no valid mean (e. g. , 28 days) has been found. No clear relationship between fertility and a relatively narrow range of fluctuation in length of cycles has been detected. Fertile matings continued to be obtained in all months (in contrast, see report on matings in the field colony). Some observations on matings, gestation, birth and postnatal development were published in 1960 (see reference 20).

Collection of hematological data has continued, and an effort is currently being made to assemble and publish newly available information on the blood picture and blood chemistry of "normal" rhesus monkeys from another institution. Collection of information on other biological values at known ages is in progress.

Collaborative studies in physiology and biochemistry of fetal and newborn monkeys, initiated last year by Dr. Geoffrey Dawes and other visiting members of the Nuffield Institute for Medical Research, were continued during the summer of 1960. Dr. H. N. Jacobson was assigned to the Oxford laboratories for three months last spring to participate in analysis of the 1959 data, and to help organize new plans for the work in Puerto Rico in 1960. Twenty-nine timed matings were obtained for the

latter studies. Reports of the 1959 aspects of the investigation are contained in references 17, 18, 21 and 27.

Monkey infants, born naturally and reared in an incubator at 30°C during the first two weeks, showed an almost two-fold increase in minimal oxygen consumption at neutral temperature (which was found to be 35°C ± 1.5°C). However, others reared at 35° C, show the same increase in minimal oxygen consumption as those reared at 30° C, and it seems unlikely that the change in temperature occurring at birth is an immediate factor in determining increase in oxygen consumption.

Measurements were made of blood pH, lactic acid, blood sugar, and of tissue carbohydrate concentration of heart, lung, liver, muscle, and brain in fetal monkeys of 60 or more days' gestation (full term is 168 days). There are very large changes in the carbohydrates reserves of the different tissues. These quantitative data await analysis in terms of the events of development and birth. Similarly, data on potassium and sodium content of body fluids were collected, and analysis is in progress.

Data on heart rate and the electrocardiogram of normal monkeys at birth (including premature birth) and throughout postnatal life are being collected, but have not been analyzed. Some information on blood pressures has been obtained.

Study of normal behavior of newborn, infant and young laboratory monkeys is still in progress and comparison with behavior of monkeys born in a natural habitat is being made. The neurological status of animals throughout postnatal life is undergoing study. Data on maturation of motor patterns, reflexes, and the sensorium, are being acquired.

Maturational changes in the normal monkey electroencephalogram (unipolar and bipolar leads) has been described by Dr. Marisa Robert de Ramirez de Arellano, Chief of the Section on Experimental Neurology (references 9, 31, 35). At birth the EEG of the awake monkey shows generalized low-voltage, irregular waves of 1-3/sec, with some 8-10/sec activity superimposed. During sleep, parietal leads give some high-voltage slow waves and paroxysmal bursts of 5-6/sec sinusoidal waves, with spread to other levels. These are considered to be comparable with the "sleep spindles" of the human EEG. The occipital leads give slow waves of lower voltage.

During the first month, the EEG of the awake monkey shows an increase in amplitude, particularly in frontal and parietal leads. While asleep, the voltage in all leads becomes greater about the end of the first week; and the parietal spikes are so marked at the end of two weeks that they could easily be thought to be seizure discharges.

During the second to sixth months, the generalized low-voltage, slow activity in the waking state increases rapidly in amplitude and shows the characteristic pattern of generalized high-voltage sinusoidal waves. Another pattern of spindle formation, 15/sec waves of moderate voltages, frequently appears during spontaneous sleep.

Photic stimulation proved to be very effective in driving the occipital cortex after the first few days when the stimulus frequency coincided with the dominant frequency of the EEG at any particular age. Evoked activity of highest voltage occurred. Marked changes in the EEG were encountered in a few animals during photic stimulation. Four deaths occurred during periods of photic stimulation in which the EEG showed these profound changes. This finding is being pursued further.

The EEG of a group of monkeys asphyxiated and resuscitated at birth is a subject of current study and comparison with the EEG of normal animals of comparable ages will be drawn.

The first objective of the project in Puerto Rico, to ascertain the role of asphyxiation during birth in producing neurological disorders in the offspring, has been achieved. Details of the procedures used, and the principal results were reported in 1958 and 1959 (see references 9, 13, 16, 23, 26, 28). All degrees of neuromotor and psychological handicaps have been encountered in infant monkeys asphyxiated and resuscitated at birth. The motor deficits have ranged from transient weakness to complete incapacitation persisting as long as the animals have been permitted to live. Varying degrees of spastic paralysis, ataxia, dyskinesia, tremor, and motor seizures have been encountered; some of the young monkeys have closely resembled human beings with "cerebral palsy". Sensory defects have been less obvious and more difficult to test for than motor defects, although deafness (partial or total), and vestibular abnormalities have been recognized. Those not having permanent motor and sensory deficits, nevertheless have differed significantly from controls in respect to reactivity, emotionality, amount of locomotion, and manual dexterity when observed in psychological test situations at 10 to 14 months of age, (references 10, 11). The brains of all animals thus far studied histologically have exhibited structural changes; these were reported in 1959 (see references 13, 16, 28, 29). A constant pattern of damage has been consistently encountered, varying in severity more or less with the severity of asphyxiation during birth.

New objectives are to standardize the physiological conditions during the asphyxial process and to obtain measurements of damage under agreed criteria; and in these animals to seek ways to prevent the damage to the nervous system from occurring by introducing appropriate

therapeutic measures. Dr. Dawes and his associates began one aspect of the study recently. They asphyxiated 10 fetal monkeys for 15 minutes upon delivery by section under local anesthesia and resuscitated them as in previous experiments. However, 5 were given an infusion of sodium carbonate and glucose at a rate sufficient to prevent the arterial pH (sampled every 5 minutes) from falling. This form of treatment improved the chance of survival, as judged by respiration, blood pressure and heart rate. The infused animals recovered more rapidly than the uninfused animals during resuscitation. Some of the monkeys are still living, and the brains of all will be examined for evidence of cerebral damage. Preliminary findings in one infused monkey indicate that the characteristic pattern of neuropathology was not prevented, but the possibility exists that a difference in degree of severity of the defect may be encountered when all speciments have been studied.

Experiments involving asphyxiation of newborn and adolescent monkeys by nitrogen gas have supplemented those in which asphyxiation was induced in the fetus in amnio. A marked difference between the two groups was found. Even after nitrogen asphyxiation was carried to the point of cardiac arrest, which required opening the chest and cardiac massage, the pattern of brain damage that had been encountered after asphyxiation in amnio was not found. The heart of the newborn and young adult monkey appears to be less resistant to asphyxia than the brain. Cardiac resistance to asphyxia decreases from late fetal to adult life, possibly due in some measure to a reduction in cardiac glycogen reserves.

Experiments involving complete hemidecortication of infant monkeys were initiated by Drs. Max Ramirez de Arellano and C. M. Combs. These have demonstrated that the monkey has a remarkable capacity to compensate for loss in early postnatal life of one-half of its cerebral cortex. Within a few weeks after the surgery, the young animals showed so little motor defect that only the most critical observation would reveal it as they walked about and climbed. The homonomous hemianopsia and other sensory losses were readily determined. Other investigators have described marked compensation by adult monkeys after hemispherectomy, but there appears to be a difference in compensation between the very young monkey and in the adult.

A number of incidental observations on monkeys have suggested fields for study in the future. For example, hemolytic anemias have been encountered in several. One of these delivered a postmature fetus about 48 hours after it had died in utero, and then went into a condition closely resembling human eclampsia. Dr. G. Dixon, visiting scientist from the University College of the West Indies, performed a few experiments, producing hypertension and endometrial changes suggestive of preclampsia.

Minor teratological abnormalities have been found on rare occasions in newborn monkeys. Twinning has not been encountered in the laboratory colonies. Little noteworthy spontaneous pathology has occurred: meningo-encephalitis in one malnourished newborn infant, one questionable case of pulmonary carcinoma in a mature female, and a few pneumonias. One adult female spontaneously developed a rapidly progressing venous thrombosis and died when the clot reached the heart. One breech delivery with retension of the fetal head resulting in asphyxiation (resuscitated, and exhibited the characteristic neurological defects) was observed. Diabetes mellitus, has been encountered in old monkeys. A coronary occlusion took place in one fetal monkey during physiological recording of blood pressure and the electrocardiogram.

Investigations in the field. The staff at Cayo Santiago now consists of Dr. Carl Koford, Chief of the Section of Primate Ecology, Dr. Clinton Conaway, from the University of Missouri, Visiting Scientist for 6 months, and Mr. Carlos Nagel. The extramural project under Dr. James Gavan, of the Medical College of the State of South Carolina, is being conducted by two technicians. Reproduction, social organization and population dynamics in the free-ranging colony of Macaca mulatta constitute three main facets of study.

The breeding season on Cayo Santiago remains about the same as in previous years. Births began in late January and extended until June, with the peak in the middle of March. Approximately 70% of mature females of breeding age conceived and gave birth to 72 infants (including 4 stillborn). This year the sex ratio was 3 females to 2 males, in contrast to 3 males to 2 females in 1959. The net increase of births over deaths on Cayo Santiago this year was approximately 50 animals.

The 5 social groups on Cayo Santiago that were present in 1959 persisted as such until the summer of 1960, when one split into two; there are now 6 social groups on the island, varying the number from 26 to 100 monkeys per group. One group maintains a territory on the small peninsula, and although making forays to the main part of the island, returns at night. In the case of the other 5 groups occupying the main part of Cayo Santiago, there is a tendency to move about territorially during the daytime, but for a number of days at a time each group may maintain night quarters in a particular region. Up to this year, immature males were the principal animals shifting from one group to another. This year, for the first time 3 dominant adult males shifted from one group to another. Not only is there an order of dominance within each social group, but there is an order of dominance between social groups; the most dominant group has a prior position at the feeding station.

With Dr. Conaway's arrival, it was possible to make a more thorough study of the "estrous cycle". This, we define as the period during which the female is receptive to the male and seeks his attention. With it there are color changes and mild swelling of the sex skin and the female becomes nervous. Variability in length of "estrous cycle" appears to be usual, just as variability in the menstrual cycle is the rule. The earliest "estrous cycles" this year appeared in late July; some animals are expected to be in "estrus" as late as the middle of January. This seasonal breeding behavior of the monkeys on Cayo Santiago is in contrast with that observed in caged colonies at Bethesda and San Juan, where no seasonal peak in a number of fruitful matings had been noted.

Dr. Conaway has made testicular biopsies on approximately 20 male monkeys, and an experiment involving androgen implants has been started.

The colony on Cayo Santiago provides a unique opportunity to study social behavior in a group of animals that are rather closely interrelated. One can study interaction of animals with close genetic relations; for example, a mother and the young of four previous years can be followed as a social unit. Little can be said about the relation of father to offspring, because the phenomenon of monogamy is rarely seen. The concept, outlined by Dr. Ray Carpenter when the colony was first established, of one dominant male consorting with one female for a prolonged period of time, has proven to be exceptional.

The five oldest females on the island have been removed for studies on the process of aging. One of these is a diabetic, the condition being controllable with administration of insulin. This animal is still menstruating, and it is hoped to obtain offspring from her, although she is more than 22 years old.

The observations in progress in the field will require considerable time. It is planned to remove a number of males from the island in order to control or reduce fighting and injuries, and to improve the breeding potential.

Two new islands in the Parguera area are being acquired by lease. Isla Guayacan has approximately 40 acres of high ground, and Isla Cuevas has approximately 80 acres. It is planned to develop free-ranging colonies on these islands for studies of social behavior and population dynamics in Macaca mulatta, and to introduce other species of primates, probably the tree shrew, Tupaia, and the squirrel monkey. It is essential to laboratory investigations to learn about the "normal" life of Macaca mulatta to provide a control for behavioral studies under caged conditions. Eventually it is

expected that monkeys reared without mothers, and monkeys affected in one way or another by experimental procedures, will be placed together in a "natural habitat" with others born in the wild, and their interactions will be studied. The acquisition of islands may make this possible.

Dr. Koford had an opportunity to visit the Russian Medical Biological Station (primate research) at Sukhumi in June, and was invited to talk to the group there on activities of the Laboratory of Perinatal Physiology. Excerpts from this report are included here as Exhibit "B".

Exhibit "A"

Publications from the Laboratory of Perinatal Physiology

1. Windle, W. F. : Neurological and psychological deficits from asphyxia neonatorum. Public Health Reports, 72: 646-650, 1957.

2. Windle, W. F. (editor): "Neurological and Psychological Deficits of Asphyxia Neonatorum". xiv + 336 pp. , Springfield, Illinois, Charles C Thomas, 1958.

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4. Ranck, Jr. , J. B. : "Neurological Deficits of Asphyxia Neonatorum in Monkeys", a motion picture. Anat. Record, 130: 470, 1958.

5. Ranck, Jr. , J. B. and Windle, W. F. : Asphyxia neonatorum in Macaca mulatta. Physiologist 1: 63, 1958.

6. Altmann, S. A. : How to predict what an animal will do next. Anat. Record, 131: 527, 1958.

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8. Combs, C. M. , Frontera, J. G. , Jacobson, H. N. , McCroskey, D. L. , Ramirez de Arellano, M. I. R. , Saxon, S. V. , Stiehl, W. and Windle, W. F. : "A Condition Resembling "Cerebral Palsy" in Young Macaca Mulatta Surviving Asphyxia Neonatorum". A motion picture. Anat. Record, 133: 263, 1959.

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14. Bailey, C. J. and Windle, W. F.: Neurological, psychological, and neurohistological defects following asphyxia neonatorum in the guinea pig. Exp. Neurol., 1: 467-482, 1959.

15. Combs, C. M. and Saxon, S. V.: Evoked potential evidence for connections from the cerebellar hemispheres to the sigmoid gyri. Exp. Neurol., 1: 583-591, 1959.

16. Windle, W. F., Jacobson, H. N., Robert de Ramirez de Arellano, M. I., and Combs, C. M.: Structural and functional sequelae of asphyxia neonatorum in monkeys (Macaca mulatta). Research Publ. Ass. Research Nervous Mental Disease, (in press).

17. Dawes, G. S., Jacobson, H. N., Mott, J. C. and Shelley, H. J.: Some observations on the rhesus monkey in utero. J. Physiol., London, 150: 3-4P, 1960.

18. Dawes, G. S., Jacobson, H. N., Mott, J. C. and Shelley, H. J.: Some observations on foetal and new-born rhesus monkeys. J. Physiol., London, 152: 271-298, 1960.

19. Altmann, S. A.: The social behavior of anthropoid primates. In: "The Roots of Behavior", E. L. Bliss, editor (in press).

20. Jacobson, H. N. and Windle, W. F.: Observations on mating, gestation, birth and postnatal development of Macaca mulatta. Biol. Neonatorum, 2: 105-120, 1960.

21. Shelley, H. J.: Tissue carbohydrate reserves in foetal and new-born lambs and rhesus monkeys. J. Physiol., London, 151: 24-25P, 1960.

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23. Jacobson, H. N. and Windle, W. F.: Responses of foetal and newborn monkeys to asphyxiation. J. Physiol., London, (in press).

24. Dennery, J. M. and Combs, C. M.: An evoked potential study of connections between the diencephalon and the sigmoid gyri. Anat. Record, 136: 181, 1960.

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28. Windle, W. F.: Anoxia and asphyxia: Experiments in fetal and neonatal monkeys. In: Symposia of the Houston Neurol. Soc. (in press).

29. Windle, W. F.: Discussion "Central Nervous System and Behavior". Josiah Macey, Jr., Foundation. (in press).

30. Windle, W. F. and Ranck, Jr., J. B.: Asphyxial tolerance by rhesus monkeys. Physiologist, 3: 178, 1960.

31. Robert de Ramirez de Arellano, M. I.: Maturational changes in the normal monkey EEG. Physiologist, 3: 47, 1960.

32. Combs, C. M. and Dennery, J. M.: Cerebello-cerebral connections in the monkey. Exp. Neurol., 2: 613-622, 1960.

33. Dennery, J. M. and Combs, C. M.: Diencephalic projections to the sigmoid gyri in the cat. Exp. Neurol., 3: 1961, (in press).

34. Ranck, Jr., J. B. and Windle, W. F.: Asphyxiation of adult rhesus monkeys. Exp. Neurol., 3: 1961. (in press).

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36. Vollman, R. F.: Quantitative Definitions of Eumenorrhea, Oligomenorrhea, and Polymenorrhea. Amer. Soc. for the Study of Sterility, April, 1960.

Exhibit " B "

Excerpts from Travel Report on Visit to Russian Primate Colony,

by

Dr. Carl Koford, Chief of Section on Primate Ecology

The purposes of the visit to Sukhumi were: (1) to obtain general information of the physical plant, personnel, and scope and methods of research and (2) to investigate the extent and success of the program of raising monkeys in large open yards, and exchange related ideas based on our monkey colony on Cayo Santiago.

The "Institute" was situated on a steep hillside behind the small railway station, about 5 blocks from the hotel. Public visitors had to walk up a long flight of stairs to reach Headquarters and the gate where they paid a fee for a guided tour of the monkey cages and yards. The grounds were spacious, about 60 acres in area, and the buildings widely spaced among trees and gardens. The largest buildings measured about 30 by 100 feet and were two stories in height, but all were unpretentious and furniture was plain and scant. In spite of the warm humid weather there were no electric fans or air conditioners. Large sketches of Pavlov and other Russian scientists hung on the walls. The Scientific Director, Boris A. Lapin, had recently returned from scientific meetings in Stockholm. He was very friendly and informal. He said he spoke German, but neither French nor English, so Mrs. E. M. Cherkovich, nerve physiologist, became my guide and interpreter. Both assistant directors were women, she told me.

According to Lapin, the Institute was commenced in 1927 for studies of endocrinology, and from the outset monkeys were kept in large open yards. At present there were about 1400 monkeys, approximately half of them rhesus macaques and one-third Hamadryas baboons. Other species included Anubis baboons, mandrills, geladas, green monkeys, Cebus species, and Macaca irus, nemestrina, and speciosa. In addition there were hybrids, including about 30 M. mulatta X M. irus, 6 Papio hamadryas X P. doguera, 3 M. mulatta X P. hamadryas, and 3 M. nemestrina X M. mulatta.

In the southern section of the grounds, near headquarters, was a 100 ft. square cage of rusted wire used to hold weaned rhesus monkeys and baboons 6 to 12 months of age; this cage was unoccupied during my

visit. Nearby there were two baboon yards, each about 1-1/2 acres in size and surrounded by vertical concrete walls approximately 15 feet high. One yard held an adult male, 33 female, and 15 young hamadryas baboons, and an adjacent yard held a slightly greater number. Often animals were added or removed, so that the numbers in a yard were not constant. Many of the females were bred in cages before being released in the yards. The principal function of the yards was to hold the animals, but some breeding occurred there. The animals were fed brown bread, carrot tops, potatoes, oranges, or other fruits and vegetables three times a day. During my visit the food was simply strewn on a walk through the yard. There were no studies of organization, dominance, population ecology, or other social relations in these groups. However, one student had written a thesis, not yet published, on sound communication in macaques and baboons.

Near the northwest corner of the grounds there were three additional walled yards one to two acres in size, and an unused old wire-fenced yard. One walled yard held approximately 100 M. mulatta said to have arrived from China in May, 1960. Among these were several stump-tailed macaques (M. speciosa). Inside the enclosure were a dozen broadleafed trees about 30 feet in height; these were kept leafless by the macaques. Another yard held macaques brought from China in 1953; and the largest yard held more than 100 macaques of mixed species and ages. In all there were about 400 macaques in yards. Each yard contained only one adult male.

In charge of the animals in the yards and cages was Mr. Y. G. Kondakov, who had held that position for many years. He told me that baboons gave birth at all seasons, but that rhesus monkeys gave birth primarily during the period from March to July, whether in cages or open yards. He also said that male baboons became fertile at 5 years of age, and rhesus monkeys at 4 to 5 years. In his opinion, one male was sufficient to breed about 30 female macaques or baboons in the yards. But in cages the usual method was to keep about 4 females with one male for several weeks (or, to produce hybrids, for months). Recent records showed rhesus monkey births as follows: 1958, 69 females and 62 males; 1959, 71 females and 69 males.

At various sites there were barred cages. One about 50 by 20 feet in size held 20 subadult and adult male baboons; these were said not to fight in the absence of females. In another large cage there was one male with 15 female Hamadryas baboons, presumably for breeding. In one cage I saw a dozen pig-tailed macaques, and a few hybrids. There were 20 juvenile baboons in one display cage, and from 2 to 6 monkeys in each of about 10 smaller cages for public display.

Research was conducted at six separate laboratories.

In the south central section of the grounds was the laboratory of Physiology and Pathology of the Higher Nervous System, which was started in 1934. Personnel included Prof. (Mrs.) Lagoochina, 4 junior investigators, 5 assistants, an engineer, and several service women. Approximately 40 monkeys were being used in experiments, most of which involved classical Pavlovian conditioned reflexes. I watched one baboon in a 12 foot square soundproof room being trained to respond to a buzzer, a red or white light, electric shock, and display of a toy, by pushing a lever, pulling another, or operating both in proper sequence. Under study were speed of learning, influence of one response on others, loss and recovery of reflexes in operated animals, and effects of radiation. Three macaques had electrodes implanted in the brain. Of electronic instruments I saw but one oscilloscope and one moderate-sized electroencephalograph. Four baboons had open stomach fistules (the "small stomach" operation) for study of digestive contractions and juices in conditioned and unconditioned reflexes. There was apparatus for measurement of oxygen consumption and calorie production in large animals.

The chief research of Dr. Cherkovich was production of neuroses in monkeys. With adult males the procedure was to keep one in a cage next to a certain female for a long period and then substitute a male for the female. Females and young were made neurotic by changing the diurnal routine, such as putting them on a schedule of 6 hours of light followed by 6 of dark, or feeding them only in the dark (published 1959). Neurotic monkeys lost weight and hair, and also developed hypertension, coronary insufficiency, and high blood pressure. In reflex tests they showed inhibition, restlessness, stereotyped cage movements, and strange mannerisms. It was also found that neuroses could prevent menstrual cycles for half a year or more. Much of the psychological work was tedious. About two years were required to produce neuroses, and baboons were trained daily for more than 4 months before irradiation.

The Laboratory of Oncology, located in the southwestern section of the grounds, was also organized in the early 1930's. Its official chief was Dr. Petroy, who was over 80 years old and did not reside at Sukhumi. The principal work was experimental production of tumors in monkeys, rabbits, and mice through implantation of radioisotopes in bone marrow. Tumors were difficult to induce, and development required from one to 10 years. Transplantation had been unsuccessful. Tumors were studied by X-ray and histological examination. Many were preserved entire in alcohol. The same building housed the Laboratory of Biology, which was largely concerned with the establishment of biological standards of blood, temperature, cranial growth, teeth, and other characteristics of rhesus monkeys and baboons. Chemical analyses were made of sugar, cholesterol, calcium, and other blood substances, and of ketosteroids in urine. Recently there

had been such work on the functional and morphological regeneration of normal and cirrhotic liver.

In the northern section of the grounds was the Laboratory of Radiobiology, started in 1957. The chief, L. F. Semenov, told me of their studies of the pathology and prevention of radiation sickness. Genetic effects were not investigated. Animals were irradiated with gamma and X-rays, and changes in blood, endocrine glands, liver, reproductive organs, and other tissues were studied. A small separate building housed a water pool into which a chamber, containing animals, could be lowered to a known distance above radioactive cobalt located at the pool bottom. Dosages of 3 to 100 r per hour were given by this method. In a smaller building, radioactive bars were raised around cages of guinea pigs and white mice, in order to give low radiation dosages. Certain drugs given before radiation allowed 70% of the animals to survive an otherwise lethal dose. Radiation of the facial area caused sickness at much lower dosage than radiation of the brain (Semenov and Fedozov, published 1959). I saw a row of a dozen barred cages, each containing 2 or 3 monkeys which had been irradiated two years earlier with different dosages in different parts of the body. Some of the animals had severe radiation sickness.

In an adjacent building was the Laboratory of Infectious Diseases, where the principal research was experimental production of dysentery and other intestinal diseases. Also in that area was the Laboratory of Pathology and Anatomy, under Lapin and his assistant Mrs. L. A. Yakovleva. They had examined approximately 2000 monkeys in studies of comparative spontaneous pathology, and they showed me a display of about 200 pathological specimens in alcohol. There were many tumors of intestinal and reproductive tracts, and various heart deformities. Nematodes had been found in the heart, testis, and other organs. Cestodes and microfilaria occurred in monkeys from China. Some animals had mites in the lungs. An old monkey had cataracts in both eyes. The results of the autopsies had been compiled in a thick manuscript.

As to aged animals, the records showed one female Hamadryas baboon had died at 33 years of age. A male rhesus monkey lived to 27. Still living was a 26 year old male, but he was an unsuccessful breeder. Females were normally utilized in experiments before they reached old age, but one (still living) was pregnant at 23 years. Three others had died at age 19 to 24 years.

On the final day of my visit I gave a talk, illustrated by color slides, concerning the work of the Laboratory of Perinatal Physiology at Cayo Santiago. The meeting was attended by about 75 people, most of them women in lab. coats. After my talk they asked many questions concerning

the weather, methods of capturing, handling, counting, and marking monkeys, monkey food, who did various jobs, and other simple factual matters. They seemed amazed that we used so few technicians and service men, for helpers were abundant at Sukhumi. They were intensely interested in our project, and they had not heard of it before. Prof. Lapin cordially thanked me. Later he presented me with two books and with photographs of Sukhumi and the Institute. He requested especially the names and addresses of other centers of primate research (he had not heard of the Southwest Foundation for Research or the Japan Monkey Center). In general, the scientists of the Institute were poorly informed regarding primate work in other parts of the world.

Lapin expressed interest in visiting Puerto Rico and asked me about travel expenses. Either he or Dr. Cherkovich would profit greatly from such a visit. If an American scientist worked at Sukhumi he would find little scientific equipment available, I think. But he would find the atmosphere informal and friendly, quarters spacious, service help plentiful, animal subjects abundant, and many lines of research untouched.

TABLE OF ORGANIZATION OF INTRAMURAL RESEARCH

(Personnel on Hand November 1960)

Office of Associate Director:

Associate Director -- G. Milton Shy, M.D., M.S., M.R.C.P.
Clinical Director -- Maitland Baldwin, M.D., M.S., F.R.C.S.
Administrative Officer -- Elliot Brookman, B.A.
Secretary -- Mary B. Calley
Secretary -- Maxine O. Reynolds
Clerk -- Joyce A. Jenkins
Clerk-Typist -- Greta G. Albrecht

Medical Neurology Branch:

Chief -- G. Milton Shy, M.D., M.S., M.R.C.P.
Associate Neurologist -- W. King Engel, M.D.
Medical Officer -- Bushnell Smith, M.D.
Medical Officer -- Forbes H. Norris, Jr., M.D.
Medical Officer -- Charles A. Payne, M.D.
Clinical Associate -- Francis L. Merritt, M.D.
Visiting Scientist -- Marco Mumenthaler, M.D.
S. A. Surgeon -- Paul H. Altrocchi, M.D.
S. A. Surgeon -- Donald H. Silberberg, M.D.
S. A. Surgeon -- Edward L. Eyerman, Jr., M.D.
S. A. Surgeon -- Daniel B. Drachman, M.D.
S. A. Surgeon -- David A. Drachman, M.D.
Medical Technologist -- William B. Matthews, Jr., B.S.
Clerk-Stenographer -- Uva W. Cable
Clerk-Typist -- Harriett C. Ottenstein
Medical Technician -- Guy G. Cunningham

Section on Biophysical Applications:

Physicist -- Donald J. Cummings, Ph.D.
Electronic Development Technician -- Lawrence C. Bromery

Section on Clinical Applied Pharmacology:

Pharmacologist -- Richard L. Irwin, Ph.D.
Physiologist -- Manfred M. Hein, B.S., M.S.
Chemist -- Henry J. Smith, III
Clerk-Stenographer -- Betty J. Jenkins

Section on Neuroradiology:

Visiting Scientist -- Giovanni Di Chiro, M.D.
Clerk-Stenographer -- Constance Ronsisvalle

Section on Clinical Neurochemistry:

Neurochemist -- Donald B. Tower, M.D., Ph.D.
Physiologist -- Beni M. Horvath, M.D., Ph.D.
Chemist -- Michael B. Sporn, M.D.
S. A. Surgeon -- Charles W. Dingman, Jr., M.D.
Chemist -- Edmund L. Peters
Physical Science Technician -- Joseph B. Proctor
Secretary -- Virginia L. Gray
Clerk (Typing) -- Raymond G. Humphrey

Surgical Neurology Branch:

Chief -- Maitland Baldwin, M.D., M.S., F.R.C.S.
Associate Neurosurgeon -- Choh-luh Li, M.D., M.S.
Associate Neurosurgeon -- John M. Van Buren, M.D., M.Sc.
Medical Officer -- Edward J. Laskowski, M.D.
S. A. Surgeon -- Joseph J. Adamkiewicz, M.D.
S. A. Surgeon -- Don A. Maccubbin, M.D.
S. A. Surgeon -- Richard J. Otenasek, M.D.
S. A. Surgeon -- Philip J. Ferris, M.D.
S. A. Surgeon -- James P. Gills, M.D.
Photographer -- Fred H. Meiller
Photographer -- Wesley Pearson, Jr.
Secretary -- Dolores B. Thompson
Clerk-Stenographer -- Ruth C. Jordan
Clerk-Dictating Machine -- Helen M. Andregg
Clerk-Dictating Machine -- Myrtle Ruth Smith

Section on Primate Neurology:

Nurse Supervisor -- Frances D. S. MacDonald, R.N.
Staff Nurse -- Mary T. Birmingham, R.N.
Staff Nurse -- Delta H. Trickett, R.N.
Staff Nurse -- Joyce C. Kegel, R.N.
Staff Nurse -- Mildred L. Haddox, R.N.
Medical Technician -- Laura S. Arakaki
Medical Aid -- James R. Waldschmidt
Medical Aid -- Morris M. Williams
Medical Biology Technician -- Norman E. Mills
Medical Biology Technician -- George R. Duvall
Physical Science Aid -- Myron W. Robertson
Clerk-Typist -- Celeste L. Sewell
Animal Caretaker -- Otis A. Prince
Medical Biology Technician -- Adrian P. Loftis
Medical Aid -- Daniel Alfonso
Medical Aid -- Jimmie L. Scott
Medical Aid -- Octavie W. Jacobs

Section on Neuroanesthesiology:

Visiting Scientist -- Anibal H. Galindo, M.D.
Electronic Development Technician -- William P. Edwards
Physical Science Aid -- Ralph E. Meadows

Section on Clinical Psychology:

Visiting Scientist -- Herbert C. Lansdell, Ph.D.
Psychologist -- Nelly M. Urbach
Psychologist -- Jean E. Hunter
Audiologist -- Sylvia W. Jones

Section on Clinical Neuropathology:

Visiting Scientist -- Igor Klatzo, M.D., M.Sc.
Visiting Scientist -- Jaime Miquel, Ph.D.
Supervisory Medical Technician -- Joseph T. Walker
Secretary (Typing) -- Arlene E. Keener
Biologist -- Felecia T. White
Histopathology Technician -- Naomi J. Mims
Histopathology Technician -- Frances B. Mays

Section on Developmental Neurology:

Visiting Scientist -- Anatole S. Dekaban, M.D.
Biologist -- Margaret L. Barbee
Medical Technician -- Joan D. Crowe
Secretary -- Phyllis P. Shimonkevitz

Ophthalmology Branch:

Chief -- Ludwig von Sallmann, M.D.
Visiting Scientist -- Gerald van Alphen, M.D.
Visiting Scientist -- Hubert HÜckel, M.D.
S. A. Surgeon -- Edward Okun, M.D.
S. A. Surgeon -- Richard B. Oglesby, M.D.
S. A. Surgeon -- Ottiwell W. Jones, M.D.
S. A. Surgeon -- Kenneth A. Simon, M.D.
Secretary -- Dorthy L. Evans
Clerk-Stenographer -- Nancy L. Atwood
Clerk (Typing) -- Harriett B. Murphy
Clerk-Stenographer -- Janet C. Dettmers
Clerk-Stenographer -- Naomi K. Boetker
Medical Aid -- Joan L. Coble

Section on Ophthalmology Chemistry:

Chemist -- Robert A. Resnik, Ph.D.
Chemist -- John B. Wolff, Ph.D.
Chemist -- Esther E. Coldren

Section on Ophthalmology Physiology:

Medical Officer -- Peter Gouras, M.D.
Physicist -- Ralph D. Gunkel, O.D.
Chemist -- Patricia A. Grimes
Biologist -- Sarah L. Robinette
Electronic Development Technician -- James L. Jones

Section on Cell Biology:

Visiting Scientist -- Sjoerd L. Bonting, Ph.D.
Biologist -- Naomi M. Hawkins
Biologist -- Mel Rose Perry

Section on Cytology and Histopathology:

Visiting Scientist -- Theodor Wanko, M.D.
Physiologist -- Leo L. Caravaggio
Biologist -- Mary Ann Gavin
Histopathology Technician -- Eleanor M. Collins
Biologist -- Marie E. Merriman
Medical Technician -- Kirk Weaver
Histopathology Technician -- Luther R. Dowell, Jr.

Section on Ophthalmology Pharmacology:

Supervisory Pharmacologist -- Frank J. Macri, Ph.D.
Research Technician -- Joseph G. Brown

Electroencephalography Branch:

Chief -- Cosimo Ajmone Marsan, M.D., Ph.D.
Visiting Scientist -- Arturo Morillo, M.D.
S. A. Surgeon -- Robert J. Gumnit, M.D.
EEG Technician -- Maureen B. de Lemos
EEG Technician -- Helen T. Ellerbe
EEG Technician -- Barbara A. Lightfoot
EEG Technician -- Emily B. Smith
EEG Technician -- Valerie Emery
Research Technician -- Samuel W. Cooper
Secretary -- GeorgeAnn Johnson

Section on Clinical Neurophysiology:

Secretary -- Paula B. Pavey

Laboratory of Neuroanatomical Sciences:

Chief -- Sanford L. Palay, M.D.
Secretary -- Phoebe T. Franklin

Laboratory of Neuroanatomical Sciences (Cont'd):

Clerk-Typist -- Gertrude E. Wright
Clerk-Typist -- Patricia P. Comanduras
Clerk-Typist -- Foye A. Barnard

Section on Development and Regeneration:

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Full Surgeon -- Earl R. Feringa, M.D.
S. A. Surgeon -- Larry J. Embree, M.D.
Biologist -- Jerald J. Bernstein, Ph.D.
Physiologist -- John O. Smart
Photographer -- Clarence Kuritzky
Physiologist -- Elizabeth Dorrill
Medical Technician -- Janina D. Ziemnowicz
Research Technician -- Albert V. Cantu
Medical Biology Technician -- Willie Perkins
Medical Biology Technician -- Melvin H. Carroll
Medical Biology Technician -- William N Dorsey

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Chemist -- George J. Koval
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Supervisory Physicist -- John W. Moore, Ph.D.
Physiologist -- Robert E. Taylor, Ph.D.
Physiologist -- William J. Adelman, Jr., Ph.D.

Laboratory of Biophysics (Cont'd):

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A. Surgeon -- William Knox Chandler, M.D.
Visiting Scientist -- Uichiro Kishimoto, D.Sc.
Electronic Engineer -- Leonard Binstock
Physiologist -- Ernest R. Whitcomb
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Section on Spinal Cord:

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Research Technician -- William L. Beane
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Chemist -- Eberhard G. Trams, Ph.D.
Surgeon -- Bernard Agranoff, M.D.
S. A. Surgeon -- Joseph D. Robinson, M.D.
Chemist -- Roy M. Bradley
Chemist -- Carl J. Lauter
Chemist -- Joseph V. Formica
Secretary -- Lillian L. Wease
Medical Biology Technician -- James M. Boone

Section on General Neurochemistry:

Laboratory Worker Leader -- Clifford A. Seay
Laboratory Worker -- Harold E. Smith

TABLE OF CONTENTS

NINDB- 1(c): Studies on the problem of epilepsy with emphasis on focal seizures.

NINDB- 2(c): Pathologic findings in patients with "centrencephalic" electroencephalographic patterns.

NINDB- 3(c): Pathological and electroencephalographic analysis of metastatic tumors of the brain.

NINDB- 4(c): Depth electroencephalography.

NINDB- 5(c): Cortical control of sensory imput.

NINDB- 6(c): Analysis of callosal responses in the visual cortex of cat.

NINDB- 7(c): Histopathological and chemical investigations of neuromuscular disorders.

NINDB- 8(c): Physiology and pharmacology in myasthenia gravis.

NINDB- 9(c): The use of monoamine oxidase inhibitor as an anticonvulsant medication for centrencephalic seizures.

NINDB-10(c): Cytological studies of neuromuscular phenomena in patients with neuromuscular disease and in experimental animals.

NINDB-11(c): Native state of bacterial and viral desoxyribonucleic acid (DNA).

NINDB-12(c): Electrolytes and energy metabolism in normal and epileptogenic cerebral cortex in vitro.

NINDB-13(c): Metabolism of free and protein-bound amino acids in normal and epileptogenic cerebral tissues.

NINDB-14(c): Isolation, fractionation, characterization and metabolism of brain nucleic acids (RNA and DNA).

NINDB-15(c): Muscle proteins in neuromuscular diseases.

NINDB-16(c): Study of muscle cholinesterase and its inhibitors.

NINDB-17(c): Interaction between macromolecules and pharmacologically active chemicals.

NINDB-18(c): Neurohumoral factors in cystic fibrosis of the pancreas.

NINDB-19(c): The localization of muscle fibers in a single motor unit.

NINDB-20(c): Brain scanning with radio-iodinated antifibrinogen (RIAF-encephalography).

NINDB-21(c): Effects of chronic administration of corticosteroids on the lens.

NINDB-22(c): Studies of the incorporation of H^3 thymidine in the corneal endothelium of the rabbit.

NINDB-23(c): Study on mitosis in the corneal endothelium.

NINDB-24(c): Studies of the effect of high energy proton microbeams, simulating cosmic primaries, on the mouse lens.

NINDB-25(c): Study of ocular toxoplasmosis and its therapy.

NINDB-26(c): Basic factors in refraction anomalies.

NINDB-27(c): The ocular toxicity of 1-phenyl-2-hydrazinopropane (JB-516).

NINDB-28(c): Clinical investigations on rare diseases of the eye.

NINDB-29(c): Corticosteroids as a cause of cataract in the human.

NINDB-30(c): Clinical glaucoma study.

NINDB-31(c): Studies on the activity of cells in the eye of the limulus.

NINDB-32(c): Organizational aspects of vertebrate retina determined electrophysiologically.

NINDB-33(c): Electrophysiological and photochemical relationships in the vertebrate retina.

NINDB-34(c): The adaptation of electrical enhancement techniques to clinical electroretinography.

NINDB-35(c): Design and construction of ophthalmic instruments.

NINDB-36(c): Functional studies in retinal anomalies and diseases.

NINDB-37(c): Increment thresholds in a subject deficient in cone vision.

NINDB-38(c): Rhodopsin measurement and dark adaptation in a subject deficient in cone vision.

NINDB-39(c): Dark adaptation and regeneration of rhodopsin.

NINDB-40(c): Investigations on spectral sensitivity of diurnal squirrels.

NINDB-41(c): Some aspects of electroretinographic responses in the American red squirrel.

NINDB-42(c): Some observations on the off-effect of mammalian cone electroretinogram.

NINDB-43(c): A study of the proteins of the lens.

NINDB-44(c): The structure of ganglioside.

NINDB-45(c): The rhodopsin cycle in the developing mammalian eye.

NINDB-46(c): Membrane ATP-ase and aqueous formation.

NINDB-47(c): Influence of alpha chymotrypsin on the healing of corneal endothelial wounds.

NINDB-48(c): Electron microscope study on the protozoon Toxoplasma gondii.

NINDB-49(c): Electron microscope studies on biopsies of human muscle disease.

NINDB-50(c): Electron microscopic studies on tissues of the eye.

NINDB-51(c): The role of the vasculature in the maintenance of intraocular pressure.

NINDB-52(c): Study on the pharmacodynamics of various agents affecting intraocular pressure.

NINDB-53(c): Observation of the flow pattern in the ciliary processes of albino rabbits. An in vitro study.

NINDB-54(c): Epileptogenic mechanisms in the brain of man and other primates.

NINDB-55(c): Functional representation in the temporal lobe of man and higher primates.

NINDB-56(c): Studies of involuntary movements.

NINDB-57(c): Effect of lesions upon the function and structure of the human central nervous system.

NINDB-58(c): A study of the functional anatomy and pathology of the human visual system.

NINDB-59(c): Effect of radio-frequency energy on primate brain mechanisms.

NINDB-60(c): Study of inhibitory interneurons in the cerebral cortex.

NINDB-61(c): Cortical intracellular potentials in response to stimulation of the geniculate body.

NINDB-62(c): Basic mechanism of electrical activity recorded from surface of brain.

NINDB-63(c): Basic mechanisms of single cell discharges in cerebral cortex.

NINDB-64(c): Physiological studies in tissue culture material.

NINDB-65(c): The effects of deep hypothermia in higher primates.

NINDB-66(c): Study of deep hypothermia in dogs.

NINDB-67(c): Psychological evaluation of temporal lobe operations.

NINDB-68(c): Observations on vascular permeability in the brains of rats exposed to alpha particle radiation.

NINDB-69(c): Study on pinocytosis in the nervous tissue.

NINDB-70(c): Study on morphological changes due to exposure to radio-frequency energy.

NINDB-71(c): Study of vascular permeability in the central nervous system using fluorescein labeled serum proteins (FLSP) technique.

NINDB-72(c): Histochemical study on central core disease.

NINDB-73(c): Observations on alterations in the vascular permeability in the experimental allergic encephalitis.

NINDB-74(c): Investigation of the etiology, pathology and clinical manifestations of cerebral palsy and allied conditions; also of the epilepsy during childhood.

NINDB-75(c): Maternal condition during pregnancy and the course of birth in relation to neurological abnormalities in the infants and pathologic lesions in products of abortion.

NINDB-76(c): Pathological lesions in the central nervous system occuring during prenatal, intranatal and early postnatal life.

NINDB-77(c): The incidence and the type of the central nervous system abnormalities encountered in offspring born to diabetic mothers.

NINDB-78(c): Measurements of external and internal orbital distance in males and females from birth to adulthood.

NINDB-79(c): Experimental production of congenital malformations of the central nervous system.

NINDB-80(c): Study of chromosomes in patients with congenital malformation and in mongoloids.

NINDB-NS-NC-1: Ultrastructure of nervous system.

NINDB-NS-NC-2: The role of gamma-amino-butyric acid in the metabolism of the central nervous system.

NINDB-NS-EN-3: The pathologenesis of myelopathies.

NINDB-NS-EN- 4: The functional significance of oligodendrocytes.

NINDB-NS-EN- 5: "Light" microscopic studies of normal and diseased neurons.

NINDB-NS-DR- 6: The specificity of the sympathetic nervous system in the regulation of pupillary function.

NINDB-NS-DR- 7: Heterogeneous reinnervation of the diaphragm.

NINDB-NS-DR- 8: Regeneration of peripheral nerves.

NINDB-NS-DR- 9: Regeneration in the peripheral nervous system: selectivity of pathways and specificity of terminations.

NINDB-NS-DR-10: Aspects of experimental neuroembryology.

NINDB-NS-DR-11: Regeneration in the spinal cord of cats and monkeys.

NINDB-NS-DR-12: Studies to determine the possible role of auto-immune antigens in cerebral scar formation.

NINDB-NS-DR-13: Studies of spinal cord regeneration in the newt.

NINDB-NS-DR-14: The neurological basis of color vision in the fish.

NINDB-NS-DR-15: Gustatory function following heterogeneous reinnervation of taste buds in the fish.

NINDB-NS-FN-16: Further studies on the efferent component of the vestibular nerve.

NINDB-NS-FN-17: Comparative anatomy of the afferent and efferent auditory nerve connections in submammalian vertebrates.

NINDB-NS-FN-18: The ascending and descending connections between the medial geniculate body and the auditory cortex.

NINDB-NS-FN-19: A study of the auditory and vestibular afferent and efferent systems including the receptors.

NINDB-NS-FN-20: Study of the afferent extrinsic and intrinsic neuronal connections of the medial geniculate body.

NINDB-B-1: Ionic permeabilities of the squid giant axon membrane. Electrical experiments and analyses.

NINDB-B-2: Ionic permeabilities of nerve membranes: theoretical investigations.

NINDB-B-3: Studies of acetylcholinesterase inhibition and nerve activity.

NINDB-B-6:	Ionic permeabilities of nerve membranes: investigations of new methods and of other axons than squid.
NINDB-B-7:	Ionic permeabilities of squid giant axon membrane. Tracer and electrical experiments and analyses.
NINDB-NC-1:	Biosynthesis of sphingolipids.
NINDB-NC-6:	Biosynthesis of aromatic compounds.
NINDB-NC-7:	Metabolism of inositol.
NINDB-NC-14:	Enzymatic synthesis of fatty acids.
NINDB-NC-15:	Biosynthesis of cholesterol.
NINDB-NC-16:	Role of redox reactions in excitation.
NINDB-NC-17:	The structure and the biosynthesis of brain gangliosides.
NINDB-NC-18:	Glycolipids as receptor sites.
NINDB-NC-19:	Effect of hydrocarbons on enzyme synthesis.
NINDB-NC-20:	Carboxylation of Acyl-CoA derivatives.
NINCB-NP-SS-5:	The mechanism of nerve excitation.
NINDB-NP-SC-3:	Generation of impulses in nerve cells.
NINDB-NP-SC-8:	Generation of impulses in the Mauthner cell of the goldfish.
NINDB-NP-SC-9:	Studies of chronic changes in single nerve cells associated with learning.
NINDB-NP-SC-10:	Intracellular recordings from inferior colliculus and medial geniculate in response to auditory stimulation.

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

OF THE

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

1960

INTRODUCTION

The report contained herein represents the first Annual Report of the reconstituted National Institute of Neurological Diseases and Blindness. As of this reporting period, the Institute now has been organized so that the Intramural Program contains an integrated Basic and Clinical Unit which is administratively self-sufficient. The accomplishment of this has been the result of much thought on the part of all concerned including the individual scientists, the program directors, and the Institute Directors throughout the framework of the entire National Institutes of Health. Through this period of transition, advice has been deliberately sought and gratefully received from the Board of Scientific Counselors and from many scientists both in the United States and abroad.

The Institute at the present time consists of eight integrated laboratories and branches. These are the Laboratories of Neuroanatomical Sciences, Neurophysiology, Biophysics and a single basic Section of Neurochemistry. The branches are as in the previous reports: Medical Neurology, Ophthalmology, Surgical Neurology, and Electroencephalography. Such integration of the Basic and Clinical Programs of the Institute and separation from the basic programs of Mental Health have not been without some concomitant changes in personnel. These will be reviewed in the appropriate units.

The administration of the Intramural Program has been placed under the purview of a newly created Associate Director for Intramural Research. This is the first time the Institute has had a Scientific Director. Under the administrative reconstitution of the Institute, all scientific programing will fall under the administrative purview of this Director.

The position of Clinical Director now will have the overall review, co-ordination, and administration of all research of the Institute pertaining to patient care or utilization of patient facilities. Dr. Maitland Baldwin has been appointed the Clinical Director of the Institute. Personnel changes have been minimal in the Clinical Unit with the loss of but one Senior Scientist, Dr. Michelangelo Fuortes, who returned to Milan, Italy to take over directorship of a new Neurophysiological Unit.

Many changes, however, have occurred in the basic laboratories of the Institute. Dr. William Windle is now the Assistant Director of the National Institute of Neurological Diseases and Blindness. Dr. Sanford L. Palay has been appointed Chief of the Laboratory of Neuroanatomical Sciences. The Laboratory of Neurophysiology will remain a joint laboratory between the two Institutes with the Section of Spinal Cord Physiology under Dr. Karl Frank and the Section on Special Senses under Dr. Ichiji Tasaki being assigned to the Neurological Institute. At the time of the availability of the new basic research building, these two sections will constitute the nucleus of the new Laboratory of Neurophysiology of the National Institute of Neurological Diseases and Blindness. The Laboratory of Biophysics has remained essentially unchanged. The Laboratory of Neurochemistry was split with Physical Chemistry going to the National Institute of Mental Health. Lipid Chemistry under Dr. Roscoe Brady is now assigned to the National Institute of Neurological Diseases and Blindness. It is planned that this section will be combined with the other chemical units within the Institute to form a new Laboratory of Neurochemistry. The appointment of the laboratory chief of this new Laboratory will be undertaken in the first six months of the next reporting year.

The scientific activities of the Basic and Clinical Units have joint research meetings each Monday morning involving all sections. On the second Monday morning of the month, one given section reviews for all other section and laboratory chiefs the current and anticipated research undertaken in such a section.

New facilities have also become available to the Institute in this reporting year. A building for basic research to be shared by the laboratories of the National Institute of Neurological Diseases and Blindness and the laboratories of the National Institute of Mental Health has now entered the planning stages. The completion of the initial plans for all laboratories which will fill this building have now been handed to the engineers of the Research Planning Branch of the National Institutes of Health. The new surgical wing is nearing completion, and it is visualized that surgery will start in this unit as of July, next year.

The planning of such facilities has taken much time on the part of Laboratory and Branch Chiefs involved. It has been felt by all concerned that this is an unique opportunity to be able to plan with thoroughness and foresight a research facility with such an imaginary scope as to attempt to foresee the future trends of neurological research. In such discussions, the Board of Scientific Counselors and many scientists at the National Institutes of Health, throughout the United States, and abroad have given much of their time and understanding to this program. All laboratories and branches would appear to be progressively pushing their research frontiers towards the molecular level.

Thus, the main changes will be in genetic structure and chemistry, ultrastructure, molecular chemistry, communication networks, theoretical mathematics, X-ray crystallography, enzymatic chemistry, and the biophysics of artificial membranes.

Many of these studies will be incorporated into present laboratories, but it appears to be the consensus of opinion of the scientists here and of the consultants that two new laboratories will be established within the new research facilities. These would be the Laboratories of Molecular Biology and of Neuropathology, the latter using newer techniques of ultrastructure, histochemistry, tissue culture, and immunochemistry. It is anticipated that at least one of these laboratories, i.e., Molecular Biology will be started in the coming year even though it means crowding of present facilities. Two areas of interest would be that of enzyme chemistry and genetic biophysics. Through the aid of the Dental Institute, six modules have become available to start a modest program in these areas.

The promise of a well-integrated program directed towards the further understanding of the normal and abnormal functioning nervous system is perhaps brighter than any other reporting period since the initiation of the Institute. The specific research accomplishments and projections of the individual sections may be seen on the following report which was derived from the report of the Clinical Director, the Laboratory Chiefs, the Branch Chiefs, and the Section Chiefs as well as individual surveys of every project report.

MEDICAL NEUROLOGY BRANCH

The Medical Neurology Branch has continued its efforts to investigate (1) the basic mechanisms responsible for the variety of neuromuscular disorders, (2) the detection of cerebral neoplasias, and (3) the basic metabolic abnormalities in cerebral function. Toward this end, a variety of neurophysiological techniques have been used ranging from microelectrode recordings of intracellular potentials to the physiology of the motor unit, the utilization of labeled isotopes to further understand regeneration and intermediary metabolism, the use of pharmacological agents and their synthesis, the utilization of more sophisticated radiological techniques, the use of histopathological techniques and tissue culture, and the utilization of enzyme chemistry and immunochemistry. Toward this end, 423 patients were admitted and 6,722 patient days were recorded. The average patient stay was 15.9 days, a decrease of 10 over the previous year. The number of patients so admitted was almost double that of the previous year, 373 consultations and 245 out-patients were seen. Of 423 patients, 188 suffered from some type of neuromuscular disorder.

NEUROMUSCULAR DISORDERS

In familial periodic paralysis, it was found: (1) Muscle potassium and sodium are not significantly elevated in familial periodic paralysis during attacks; (2) There is no significant change in the resting potential of paralyzed fibers; (3) During attacks, the membrane of the fiber does not conduct a propagated potential when stimulated directly; (4) The hyperpolarization apparently cannot be indicated as a cause of the paresis; (5) There is no direct relationship between the level of potassium of the serum and the attack; (6) An abnormality of glucose tolerance curve was present in all patients studied; (7) Triamcinolone causes an exacerbation of the disease, and glucagon and epinephrine induced weakness in the resting patient; (8) Aldosterone excretion, per se, was not an etiological agent of this disorder; (9) A fall in basal metabolic rate is accompanied by an exacerbation of the patient's symptoms and this is independent of TSH secretion; (10) During attacks there is an ingress of intracellular water, as seen by both the light and electron microscopes and measured chemically; (11) Such flocculations of fluid are membrane enclosed; (12) Such membranes appear to surround dilated endoplasmic reticulum; (13) A characteristic change in the mitochondria is also suggested by the electron microscope; and (14) It is suggested that granules which are present inside such vacuoles may represent the accumulation of non-diffusible intermediates in glycogen anabolism.

Incubation studies of muscle from patients with muscular dystrophy in which an attempt was made to incorporate tritiated thymidine into regenerating muscle resulted in no successful labeling. This could be because the mitotic interval exceeded the perfusion time or that mitotic division cannot occur in adult striated muscle. To clarify this, chronic osmotic infusion of tritiated thymidine has been carried out over a 3-month interval in the rat. These studies are not completed as yet.

In the late onset myopathies, two distinct programs are emerging. One in combination with the Institute of Arthritis and Metabolic Diseases has demonstrated a group of dysproteinemic myopathies associated with Sjogren's syndrome. Such patients show a typical myopathic picture clinically with gamma globulins up to 10 gm/100 cc in the serum and plasma cell infiltrations into the muscle. A study in combination with the Cancer Institute has just been initiated into the carcinomatous myopathies which, particularly in the male over the age of 55, account for approximately 80% of symmetrical muscular weakness of proximal muscles.

The study upon the family previously described in this Institute with a new disorder termed "Central Core Disease" of muscle was carried on by Dr. Franz Seitelberger, Director of the Institute of Neurology in Vienna and Visiting Scientist in the Section of Neuropathology, and Dr. Theodor Wanko from the Ophthalmology Branch. These investigators have demonstrated an abnormal content of a mucopolysaccharide within the central core. There is a decrease of lipid content in the central core and this in turn is confirmed by an electron microscopy study which demonstrates no mitochondria in the central core. A reconfirmation of the original study was found on electronmicroscopic analysis in that it was shown that the fibrils of the central core can contract differently than the remaining normal fibril.

In myasthenia gravis, studies are continuing in the clinical administration of deoxydemethyl lycoramine hydrochloride, and this now consists of a series of eight myasthenic patients. This drug is a quaternary ammonium compound with initial pharmacology worked out in the Section of Applied Pharmacology under Dr. Richard Irwin, and is derived like the Russian drug, galanthamine, from the narcissus plant. It is apparent in these eight patients that this drug is a potent anticholinesterase compound with the therapeutic level of 4.0 μg/kg. There appears to be a wider safety factor between the therapeutic level and the toxic level of this drug. The duration of action of this compound appears equal to or greater than that of pyridostigmine. It is planned to rapidly extend these studies to a larger number of patients; and if continued success is noted, then the tertiary compound will be synthesized for oral medication.

Three hundred and forty-three electrophysiological studies of muscle abnormalities were completed. It now appears that the electromyogram can detect not only the difference between myopathy and neuropathy but also between inflammatory myopathies and other myopathies, and between peripheral neuropathy and anterior horn cell disease. Nerve conduction studies have shown themselves useful in peripheral neuropathy, and repetitive nerve stimulation studies are carried out in abnormal fatigue states such as sarcoid, carcinomatous myopathy, and myasthenia gravis. Complimentary work on the contractile and non-contractile protein from biopsies removed from now over 300 muscular cases are being carried out in the Section of Neurochemistry of this Branch by Dr. Beni Horvath, and Dr. Karl G. Henriksson. With the arrival of Dr. W. King Engel as Associate Neurologist, a study has been initiated into the histochemistry of skeletal muscle as far as the distribution of several known enzymes, and tissue culture studies are to be initated both in patients and animal material. The original project undertaken by Dr. Engel is toward the histochemical localization of myoglobin within the muscle of animal and man.

Dr. Horvath and Dr. Henriksson have continued their studies with contractile and non-contractile proteins in muscular dystrophy patients. These studies are roughly divided into two groups. One is an immunological study utilizing Clam Tropomyosin A and the second is concerned with the extraction and fractional analysis of muscle protein from biopsies now obtained from over 300 cases of patients with neuromuscular disorders.

The first study is based upon the unusual immunological properties of structural muscle proteins which are reported in the previous report (cf. 59-NINDB-7(c)). The time course of antibody titers in rabbits following administration of Clam Tropomyosin A has now been followed. Injections given every other day for two weeks result in titers comparable to the highest reported in the literature for any other antigens. However, such titers would decline rapidly to low levels, with a half-life of approximately one week. Months after a titer was maximal, repeated injections of Tropomyosin A cause a rise in the titer. An attempt was made to maintain a titer by injections at repeated weekly intervals. The titer achieved and the time course of the rise corresponded to the theoretical expectation from the response of a single injection and the assumption of a single exponential decline of the titer with a half-life of approximately one week. Such a titer, however, could not be maintained but fell at about the same rate it did after single or closely-spaced injections.

Rabbits exposed to closely-spaced injections occasionally showed weight loss and death within a few months after the injections. But rabbits injected at weekly or monthly intervals regularly exhibited weight loss and death. Such animals were

autopsied and regularly showed a glomerulonephritis. However, few, if any, muscle lesions were seen in such material. It would appear that muscular necrosis from an auto-immune reaction in muscle from these experiments, at least, is not as regularly seen as are nerve lesions after nerve tissue injections as seen in the studies of Adam, et. al.

In their second study on the extraction and fractional analysis of muscle proteins, Dr. Horvath and Dr. Henriksson have taken advantage of the observation of Helander that muscle extracted with 1.1 potassium iodide (KI) extracts virtually all the proteins of the muscle. Muscle biopsies of as small a size as .017 grams to 1 gram may be utilized and with triplicate determinations as routinely done, there is the standard error of only about 0.5 mg. of nitrogen per gram tissue or 2 to 5% and that a 95% confidence limit for the regression lines calculated from the normal points vary from 0.2 to 1.5 mg. nitrogen per gram. The muscle is taken out and a homogenate is prepared from frozen tissue. This, in turn, is extracted with 0.3 cc. sodium hydroxide in the cold. From this may be determined the total nitrogen. This in turn may be then fractionated subsequently into the non-protein and protein nitrogen. The protein nitrogen in turn, by the utilization of 0.05 sodium hydroxide, may be divided into the non-collagenous and the collagenous nitrogen. This, in turn, by the utilization of potassium iodide may be divided into two groups. The non-soluble portion is practically negligible. The potassium iodide soluble protein may again be divided into two groups by the utilization of potassium chloride. Once again, one group is practically negligible, the other group which is not precipitated by the potassium chloride may again be broken into two groups by the utilization of H_2O.. That which is water-soluble, contains myoglobulin, mitochondria and other non-contractile proteins. That which is not water-soluble contains actomyosin. Utilizing such techniques, these investigators have demonstrated that the normal amount of actomyosin is present for the protein content in five different types of myopathic patients. They are now working on the water soluble fraction, i.e., the non-contractile proteins and there is some indication that this fraction may be reduced in certain neuromuscular disorders. This is of interest in that it would demonstrate the protein abnormality of muscle may not be in the contractile fibrils but in the non-contractile proteins of muscles.

The Section of Neuropharmacology has continued its studies in reference to humoral mechanisms at the myoneural junction and at the parasympathetic end-organs as well as a study on the localization of muscle fibers in a single motor unit. Dr. Richard L. Irwin has continued his work on the pharmacology of several new compounds which are derivatives of the alkaloid galanthamine. These substances have been found (as noted in the past Annual Report) to inhibit muscle, brain, and plasma cholinesterase and to increase the contraction of skeletal muscle. Of the numerous

compounds tested in this laboratory, three carried an intrinsic interest to the disorder myasthenia gravis. These were: (1) Deoxydemethyl lycoramine methiodide; (2) The dimethyl carbamate of deoxydemethyl lycoramine hydrochloride; and (3) The dimethyl carbamate of deoxydemethyl lycoramine methiodide. Deoxydemethyl lycoramine methiodide has greater cholinesterase inhibiting potency than edrophonium and differs from the carbamate compounds in having a shorter duration of action. The methiodide of the dimethyl carbamate group had the greatest potency of any of the compounds tested. Compared to neostigmine, muscle potentiation is greater and the duration of action is longer. However, toxicity after parenteral administration of this compound, as judged by LD_{50} in animals, is greater than that of neostigmine. The toxicity after oral administration, however, was less, indicating poor absorption. Atropine protected against doses which are lethal in 100 per cent of the animals. The hydrochloride of the dimethyl carbamate group had in general the same activity as the methiodide salt. However, the hydrochloride had a better absorption following oral administration. The potency of the hydrochloride in producing muscle potentiation is only 1/4 that of the methiodide, but it is still greater than physostigmine. The hydrochloride form had a delayed onset of action with approximately 30 minutes being required for maximal effect. The duration was similar to that of neostigmine. The hydrochloride has about one-tenth the toxicity after parenteral administration as did the methiodide and is only about 1/4 as toxic as physostigmine. It is the methiodide of the dimethyl carbamate form which has been utilized in clinical trials described above. A study now is in progress to determine the absorption across the blood-brain-barrier to see how much inhibition of the esterases occurs in the brain.

Many investigators in the past have postulated that excess parasympathetic stimulation of exocrine glands may be one of the mechanisms responsible for the high electrolyte and polysaccharide content of secretion in the disorder of cystic fibrosis of the pancreas in children. Direct evidence in support of this postulate, however, is lacking. Techniques developed in Dr. Richard L. Irwin's laboratory for bio-assay using the clam heart, and where feasible eserinized frog rectus muscle, were used to analyze sweat from patients with and without cystic fibrosis of the pancreas. Such patients were sweated thermally in plastic bags by the method of Di Sant Agnese for a timed period. The sweat volume, pH, sodium, and potassium were measured. Samples were acidified and used immediately or frozen to preserve activity. Dr. Edward L. Eyerman in Dr. Irwin's laboratory then determined the cholinergic activity due to a choline ester, presumably acetylcholine, and found high amounts in all but one of the 12 patients with cystic fibrosis of the pancreas in contrast to the activity obtained from non-cystic samples. These investigators have established the fact that this is a choline ester rather than choline itself. The average activity of the 12

cystic patients expressed as acetylcholine is 0.075 μg/ml. of sweat. A similar value for 12 patients without cystic fibrosis is 0.0054 μg/ml. Three cystic patients gave extremely high values averaging 0.22 μg/ml. The highest value for a non-cystic patient was 0.015 μg/ml., thus it would appear that these investigators have established that a choline ester is responsible for or associated with, the abnormal sweat patterns of mucoviscidosis.

It is now recognized and recently emphasized by Nachmanson that the majority of chemicals which alter biological functions do so by interaction with cellular macromolecular components. Such cellular components are commonly referred to as receptor sites and these have been shown to have a high degree of specificity. However, almost nothing is known as to the chemical composition of such sites either as to which cellular macromolecule is involved or what molecular or submolecular force is responsible for such interaction. Many drugs have their action at levels too low to be determined chemically and hence, must be determined by bio-assay. Such a bio-assay procedure may be used to determine whether an active substance has reacted with a macromolecule (i.e., at the receptor site). Such a study has now been initiated with certain glycolipids in which it has been determined from preliminary results that such glycolipids apparently sequester some di-quaternary compounds which have marked pharmacological effects. The biological activity of mono-quaternary compounds, however, does not appear to be changed by the presence of such glycolipids. The significance of this program to the future is great, in that an increased understanding of how drugs combine with cellular macromolecules may make predictable the development of new therapeutic agents and lead to a fuller understanding of cell function in neurological disorders.

Utilizing single axon preparations, Dr. Forbes Norris in Dr. Richard Irwin's laboratory, after electrical stimulation of such an axon, explored the remaining innervated muscle by means of glass micro-pipette electrodes. The muscle fibers supplied by such an axon were located through evoked intracellular depolarizing potentials. With this technique, Dr. Norris could then map a muscle in cross-section showing the exact location of the muscle fibers in a given motor unit. This study indicated that singly or in small groups, muscle fibers of a motor unit are widely dispersed in the muscle. The method could have selected axons of large diameter (therefore supplying larger numbers of muscle fibers than smaller axons with smaller motor units). There is still some evidence that the motor units' size is related to function, and changes such as suggested by Wohlfart of "Motor Unit Territory" could reflect a functional rather than anatomic change.

Dr. Giovanni DiChiro is continuing his work on the radiological study of the soft tissues in different muscle diseases

using high kv. ranges. The light emission of intensifying screen located in the back of the X-ray cassette is blocked with a black piece of paper placed between the screen and the X-ray film. The films obtained by such a technique are reproduced with Logetronic equipment to enhance the contrast.

The purpose of this study is to determine changes of soft tissues of the limbs in patients with different neuromuscular diseases, in particular, adipose infiltration.

GENETIC STUDIES

As noted above, chromosomal counts are being carried on in certain neuromuscular disorders in which there is a high probability of such an abnormality. In addition to this, however, the Institute has recruited a genetic biophysicist, Dr. Donald Cummings. This investigator was recruited in anticipation of the activiation of a Laboratory of Molecular Biology in the first half of the next reporting year. This investigator is now initiating studies to determine in vivo the molecular weight of bacterial and viral Desoxyribonucleic Acid (DNA). In this study, the T-2 phage will be utilized as well as the genetic material from the T-2 phage as it is extruded from the tail core into the host cell. E-coli DNA will be obtained by utilizing F+ and F- cells and causing the F+ cells to mate with the cell wall material from the F- cells, thus liberating the DNA from the F+ cell and leaving the rest of the cell intact. This extracted DNA will then be studied by a variety of physical methods, in particular, ultracentrifugation, utilizing the cesium chloride density gradient method. The molecular weight of this native DNA can then be determined from the density of the DNA and the shape of the flotation band.

It is important to know the gene size so that any molecular changes observed as part of an abnormality can be related to the genome. Thus, the estimated gene size could be used in interpretation of the genetic findings in some neurological abnormalities.

BRAIN TUMOR STUDIES

Over 690 scans utilizing radioactive iodinated albumin with the collimation device designed in this Institute has now been accomplished. The present accuracy of confirmed positives is approximately 83%. More knowledge has now been obtained as to the type of tumors from such scans, in particular, cystic astrocytomas, meningiomas, and metastatic tumors.

Dr. Giovanni DiChiro has finished his study comparing RISA encephalography and conventional neurological methods and comes to the conclusion that RISA encephalography has its main accuracy in determining the extent of certain lesions and in detection of multiple lesions such as multiple metastasis and multiple meningiomas; it is not as accurate as conventional radiology for determining the nature of different lesions, i.e., the type of tumor. Tumors missed by normal radiological methods have been detected and conversely tumors missed by RISA encephalography have been found. Dr. DiChiro comes to the unavoidable conclusion that RISA encephalography should be used together with other neurological methods and not in lieu of.

Dr. DiChiro is also carrying out scanning utilizing other radioactive labels for detection of brain tumors, in particular radioiodinated antibodies to fibrinogen. Dr. DiChiro's study suggests that sarcomatous lesions and vascular lesions of the brain may be detected more accurately with antifibrinogen than with RISA. He plans on continuing this study.

Dr. DiChiro has also finished an Atlas of Fine Encephalographic Anatomy of the Brain utilizing fractional air encephalograms. This Atlas of detailed normal pneumoencephalographic anatomy has been acepted for publication by Charles C. Thomas and Company.

Dr. DiChiro has finished his original studies in reference to the radiological criteria for the interrelation of the sella turcica and the pituitary in the coronal plane. This study is to be published in the American Journal of Roentgenology. He comes to the conclusion that in over 90 per cent of the cases it is possible to reproduce roentgenographically the width (third dimension) of the sella turcica in conventional X-ray studies of the skull. This combined with laminagraphy may establish the width of the sella turcica in 100 per cent of the cases. The adding of the third dimension of the sella turcica is the only true way in which the volume may be so determined. By using postmortem material, Dr. DiChiro has proved that the width of the sella, as determined by X-ray, corresponds to the actual width of the pituitary gland and is probably the only true measurement of the pituitary, in that the length of the pituitary does not correspond to the corresponding length of the sella.

Dr. DiChiro is also continuing his study on distribution of the major superficial veins draining the two cerebral hemispheres. His study primarily concerns the three main superficial venous channels. These are the vein of Trolard, the Sylvian vein, and the vein of Labbé. He finds the vein of Trolard more predominant on the right hemisphere and that of Labbé more predominant on the left. He has attempted to correlate this with the Wada test for hemispherical dominance and cortical stimulation at the time of operation and finds usually that in a right-

handed person that the left vein of Labbe is predominate. In over 70 per cent of the cases, the superior sagittal sinus and the sinus rectus enter into both the right and left transverse sinus. In a few cases, the deep veins enter predominately to the left transverse sinus, and the right transverse sinus takes the superficial venous drainage from the cerebral hemispheres.

EPILEPSY AND CEREBRAL METABOLISM

Studies by Dr. Bushnell Smith and Dr. Darwin Prockop have continued on the effect of a monoamine oxidase inhibitors on centrencephalic seizures. These investigators concluded from their initial studies using double-blind procedures that the monoamine oxidase inhibitors are not of value in the treatment of centrencephalic seizures. However, tangent observations during their investigation may prove of value in the understanding of such patients. Patients with centrencephalic seizures receiving monoamine oxidase inhibitors present with hyperreflexia, clonus, and confused states at levels where normal patients would exhibit no toxicity. To see where in the tryptophane metabolism this might lie, these investigators removed such patients from therapy and gave loading doses of tryptophane. The patients again presented with the same symptoms with this normal body metabolite. Hence, the centrencephalic patient appears to have some type of naturally occurring block in tryptophane metabolism. It is uncertain at this time whether this would lie at the monoamine oxidase level or at the decarbyloxase level and further studies using 5-hydroxytryptamine ingestion are now being undertaken.

Studies on cerebral metabolism are also continuing in the Section of Neurochemistry by Drs. Donald Tower, Guy McKhann and John Wherrett. These investigators are investigating the metabolism of free and protein-bound amino acids in normal and epileptogenic cerebral tissues. They have found that 40% of the total substrate oxidized by the Krebs cycle passes by way of the γ-aminobutyric acid and have confirmed this in vivo in mice using pyruvate 3-C^{14}.

The factors which regulate the channeling of the substrate by way of γ-aminobutyric acid or the alternate succinyl-CoA pathway were investigated using cerebral cortex mitochondria incubated with γ-aminobutyric acid 1-C^{14} with simultaneous measurement of the oxygen consumption and carbon dioxide evolution. In this study, they found that there was a reciprocal relationship of γ-aminobuytric acid utilization and α-ketoglutarate concentration present. If arsenite was added (this inhibits α-ketoglutarate oxidative decarboxylation), then γ-aminobuytric acid utilization is enhanced instead of depressed by high α-ketoglutarate.

If mitochondrial permeability is altered, however, then such a depression of γ-aminobuytric acid utilization by high concentrations of α-ketoglutarate was less than if intact mitochondria was used. When lower amounts of α-ketoglutarate were used, then the addition of oxidized DPN to such swollen mitochondria greatly enhanced the γ-aminobuytric acid utilization. This was not seen with intact mitochondria which are relatively impermeable to DPN.

These investigators are also continuing their studies in free and protein-bound glutamate and asparatate metabolism utilizing C^{14}-labelled amino acids. The substrate is incubated and then the isolated protein is re-dissolved and re-precipitated to constant specific activity before hydrolysis. Amino acids of the free pool and protein hydrolysate are separated chromatographically and counted. Other samples are incubated with N^{15} ammonium chloride and the various ammonia fractions liberated enzymatically (or by acid), and assayed spectrophotometrically.

These investigators found a dry protein residue of 8.4 ($\pm$ 0.5%) of the original wet weight in 36 samples of cerebral cortex. The total amide nitrogen was estimated to be 56 µM/100 mg of dry protein of which 80-90% were attributable to glutaminyl plus asparaginyl residues. The total glutamate (glutamic acid + glutamine), was about 80 µM/100 mg. dry protein. Of this, 44% was glutamine in samples immobilized immediately after excision, whereas in those slices prepared for incubation in bicarbonate-saline-glucose, only 37% was represented by glutamine. Since total glutamate does not change, it was concluded that deamidation of protein glutamine to glutamic acid with liberated amide ammonia appearing free in the tissues had occurred. This is consistent with other studies indicating protein glutamate as a major source of endogenous cerebral ammonia.

The total aspartate (aspartic acid + asparagine) was about 60 µM/100 mg. dry protein. Fifty per cent was asparagine under all conditions listed above so that no apparent deamidation of protein asparagine could be detected.

Cortical slices which were incubated in C^{14}-labelled glutamic acid or glutamine showed incorporation of the labelled amino acid into cerebral proteins in vitro. This is of the same order of magnitude as that found in vivo by Waelsch. When incubated, however, with 40 mM malonate, such slices showed a decrease of protein-bound glutamine from the control values. Such differences were of border-line significance but still may reflect a transfer of protein-glutamine amide ammonia to support additional accumulation of free glutamic acid from α-ketoglutarate under these conditions. When cortical slices were incubated with 10 mM ammonium chloride, (associated with abnormally high levels of free-pool glutamine and also with intracellular concentration of free ammonia ions), there was a

significant increase of protein glutamine at the expense of protein glutamic acid. This was not seen in the white matter and could be abolished from the cortex by intoxication with methionine sulfoximine (blocks free-pool glutamine synthesis). This suggested that the ammonium chloride effect is primarly via free glutamine (i.e., transamidation from free pool glutamine to protein-bound glutamic acid). When N^{15}-labelled ammonium chloride was used the Atoms % excess of the amide nitrogen from protein-bound glutamine was 18% of that for free pool glutamine whereas the C^{14} activity of the protein glutamine was less than 0.1% of free pool glutamine specific activity. This indicates then that the entire free glutamine moiety was not directly incorporated into protein. These investigators conclude that these findings support the concept that the amide group of protein-bound glutamine is labile and as such implies important relationships to endogenous nitrogen storage, transfers, and reactions, as well as to the "fixed" anion pool.

In their attempts to isolate protein fractions from total mixed proteins, these investigators continued their studies on a model protein, guinea pig serum asparaginase. They were able to obtain complete purification on a DEAE cellulose chromatographic column using partially purified fractions. The enzyme finally obtained represented only 1% of the total protein nitrogen applied. It had a specific activity over 2000 μM per hour per mg. protein nitrogen. This low protein concentration has prevented the usual studies of purity by electrophoresis and ultracentrifugation.

As noted above, the effect of ammonium chloride on protein glutamine amide appears to occur in the neuron. These investigators are now attempting by differential centrifugation and subsequent chromatography to determine what cellular sub-units contain the various proteins. This is done in combination with the electronmicroscopy unit of the Ophthalmologic Branch.

Dr. Donald Tower, Dr. Bushnell Smith, and Mr. Edmund Peters have continued their studies on the electrolyte and energy metabolism in normal and epileptogenic cerebral cortex in vitro utilizing the methods outlined in the previous report. Studying the effects of exogenous ammonia ions on electrolytes and fluid spaces, these investigators found that when their slices were incubated with 5-10 mM ammonium chloride, that the ammonia ions were found to compete with potassium for inward transport into the slice cells. Such a competition held at least for external ammonia concentrations of 5-10 μEq/ml., and external potassium of 5-30 μEq/ml. The free-ammonia space within the incubated slices under such conditions exceeds 100%. This clearly indicated concentration of ammonia ions within the cell against the concentration gradient. An equivalence of intracellular potassium was displaced by the ammonia. No effect, however, on the transport of sodium was noted by the ammonia ions. These investigators

felt such effects to be equally present in neurons and glial cells since the same findings were present in white matter as in cerebral cortex. Simultaneously, the non-chloride space of the neurons but not of the glial cells decreased significantly when cortical slices were incubated with the 5-10 μEq/ml. of ammonia, the average value being only 80% of normal controls, as reported in other projects. A significant fraction of the total ammonia ion transported into the neuronal cells is bound as the amide nitrogen of free and protein-bound glutamine. The extra glutamine so formed is produced by the amidation of glutamic acid and hence involves an equivalent loss of "fixed" intracellular anion. Consequent to this loss of the intracellular anion, a decrease of fluid volume, i.e., extrusion of H_2O, would be expected and is consistent with the decrease of neuronal non-chloride space observed. Under the same conditions oxygen consumption of both cortical and sub-cortial white matter was significantly depressed (an average of 16%). In addition, cortical slices exhibited a doubling of aerobic glycolysis and an equivalent elevation of glucose consumption. Samples from methionine sulfoximine treated animals behaved in an identical fashion indicating that these effects of exogenous ammonia ions are distinct from the effects on the glutamine synthesizing system. These investigators then carried their studies to sub-cellular structures, in particular, cerebral cortex mitochondria, and arrived at the conclusion that the effects of the ammonia ion under conditions in which pyruvate or α-ketoglutarate were substrates, appeared to be a direct interference with the oxidative decarboxylation of pyruvate and α-ketoglutarate, both of which utilize similar mechanisms and co-factors. The exact nature of such a block remains to be elucidated.

Electron microscopic studies seem to bear out initially the chemical conclusions in that the preliminary data indicate that those slices incubated with 10 mM NH_4Cl exhibit greater astrocytic swelling (therefore possibly a larger chloride space), swelling of the endoplasmic reticulum and possibly loss of fibrillar components of the astrocytes.

Dr. Michael Sporn and Dr. Charles Dingman in Dr. Donald Tower's laboratory have initiated a study on the isolation fractions of cellular sub-particles for studies on the characterization and metabolism of brain nucleic acids (RNA, DNA). Desoxyribonucleic Acid and RNA determinations from the whole brain and on each subcellular fraction are carried out by an adaption of the method of Scott, et. al. The samples were precipitated at 0 degrees, centrifuged with 0.4 N perchloric acid (PCA) and washed repeatedly with 0.2 N perchloric acid. The lipids were extracted twice with absolute ethanol, twice with chloroform-methanol and twice with methanol. The RNA was then extracted by hydrolysis with in NaOH at room temperature for one hour followed by precipitation of solubilized protein and DNA with hydrochloric acid. Desoxyribonucleic Acid was extracted by

1.6 normal PCA at 60 C for 7 minutes. Both R-and D-mononucleotides are measured individually by ultraviolet absorption at 260 m . Total rat brain RNA under these conditions was 1.36 (± 0.03) mg./gram wet weight and the DNA was 1.39 (± 0.06) mg/gram wet weight. These investigators plan extensions of these studies to the isolation of mitochondrial and microsomal fractions by differential and density gradient centrifugation. The purity will be assessed by appropriate specific biochemical characteristics plus electron microscopic morphological controls. The isolation of microsomal RNA will then be undertaken and compared with similarly isolated liver microsomal RNA for various characteristics.

DEGENERATIVE DISORDERS OF THE CENTRAL NERVOUS SYSTEM

The finding of a patient on the ward with typical Friedreich's ataxia and hypogammaglobulinemia allowed a unique opportunity to carry out a series of investigations to further understand the formation of excess proportions of gamma globulin in the cerebrospinal fluid protein of such patients. A number of previous cases of Friedreich's ataxia have been studied at NINDB which have shown elevated CSF gamma globulin. The initial studies of the serum and CSF protein by paper-electrophoresis, while the patient was on bimonthly injections of gamma globulin, i.e., 10 ml. intra-muscularly, demonstrated that the serum gamma globulin was 3.1% of the total protein whereas the normal is 16.6% The CSF gamma globulin, however, was 23.5% of the total protein whereas the normal is 10%. Quantitatively, as expected, the serum was calculated to contain 186 mg/100 ml. compared to a CSF content of but 5.5 mg. Thus, it was felt no conclusion could be drawn regarding these altered percentages. The gamma globulin therapy was therefore stopped for 6 months. The patient was re-examined and found to have a serum gamma globulin of only 4.4%, i.e., 273 mg/100 ml. The CSF gamma globulin was 2.5%, i.e., 0.8 mg/100 ml. This indicated immediately that the gamma globulin observed in excess proportion in the spinal fluid was not formed in the central nervous system since the CSF gamma globulin fell upon removal of exogenous gamma globulin. The patient was then injected with 40 microcuries of I^{131} labelled gamma globulin and the subsequent activity of serum and CSF gamma globulin was followed at frequent intervals. In both the serum and CSF the total activity of the sample was compared with the electrophoretic pattern (in the case of CSF after dialysis). The findings can be summarized as follows: labelled serum gamma globulin decreased after injection with a biological half-life of approximately 13.5 days; corrected for I^{131} decay. The plot, however, suggested two components, one with a half-life of 4.5 days and the other of about 45 days. The significance of two such decay rates is not clear. It may be that the more rapid half-life reflects tissue distribution while the slower represents true catabolism. There

was, however, a definite passage of the labelled gamma globulin into the cerebrospinal fluid and such activity became maximal approximately 7 days after injection. Subsequently, the CSF activity declined at a rate roughly parallel to the serum, although it was our impression that the CSF rate was slower, suggesting to some degree sequestration in the CSF compartment or a slower removal therefrom. Because of the uncertainties concerning the exact concentration of gamma globulin in the serum and CSF at lower levels, it is impossible to express the specific activities with any degree of certainty. It is therefore not certain whether the labelled CSF gamma globulin ever reached equilibrium with the serum gamma globulin . When expressed in counts per minute per milligram total protein, the activity ratio of CSF to serum never exceeded 0.435. These studies can be contrasted with Fishman's data using I^{131} labelled albumin, injected into normal dogs where the CSF activity was evident within one hour and the equilibrium time for CSF and serum S.A.(cpm/mgm total protein) was about 20 hours with the serum half-life of 6.3 days and CSF half-life of 8 days.

BRANCH OF ELECTROENCEPHALOGRAPHY

AND

CLINICAL NEUROPHYSIOLOGY

The Branch of Electroencephalography and Clinical Neurophysiology differs in essence from the other branches and laboratories in the Institute in that it carries a heavy service responsibility. A total of 1,844 electroencephalograms were performed during the past year, indicating that although the patient population of the Clinical Center has reached its ultimate level, the utilization of such techniques as this has continued to increase. A comparsion to other hospitals across the country has been pointed out by the Chief of this unit. It would appear that the service activities of this Branch belong in the upper-half as far as the monthly average of EEG's done in large hospital services throughout this country is concerned. Such hospitals usually have at least two or three electroencephalographers reporting. Here we have but one, and he is also supervising four research projects. It is obvious with the expanded utilization of this service that a second electroencephalographer must be recruited.

The research activities of the unit embrace both human and animal studies to further elucidate both normal and epileptogenic neuronal functions with particular reference to the genesis of seizures, the interaction of cortical and subcortical nuclear aggregates, the effect of expanding lesions upon the electroencephalogram, the direct recording of electrocorticograms, the electric manifestations of the cortical and subcortical substrata of involuntary movements, and the transcortical mechanisms in the study of the morphology of the "slow" electrical potentials evoked in the visual cortex by stimulation of the contralateral homologous regions.

Dr. Ajmone Marsan is continuing his studies on the understanding of the patho-physiological substrata of convulsive seizures, utilizing a combination of approaches, from routine serial EEG's during wakefulness, drowsiness, and sleep, from direct recording of the electrical activity of the exposed cortex during surgery, from direct recording of the cortical and subcortical structures in patients with chronically implanted electrodes, by eliciting seizures with systemic chemical or local repetitive electrical stimulations and finally by critical analysis of experimental data. He and Dr. John Van Buren are observing patients with temporal lobe seizures by autonomic recordings of blood pressure, pulse, skin resistance, esophageal

peristalsis, gastric motility and respiration. Many such autonomic changes appeared without electrographic correlates. On the other hand, frequently no vegetative changes were seen to accompany the onset of focal ictal activation either on the surface or the depth of the temporal lobe, although such autonomic changes may occur later as the activity increased in extent and amplitude. A sudden generalized loss of voltage or flattening of the record formed a constant warning of an imminent clinical seizure activity. In a study of the interaction of the component parts of epileptic neuronal aggregates utilizing both macro and micro electrodes, Dr. Ajmone Marsan analyzed the processes responsible for the paroxysmal cyclical firing (inter-ictal) and for the organized repetitive self-sustained (ictal) activity of such epileptical neuronal aggregates, and for the transition from one to the other. He came to the conclusion that more than just simply quantitative differences characterized such two types of activities. This author suggests paroxysmal cyclic discharges could theoretically take place in the isolated neuron, but that the self-sustained (i.e., ictal) activity could occur only in neuronal aggregates.

Controversy has existed since the middle 1940's, when Jasper and Hunter first described a paroxysmal symmetrical spike-and-wave type of discharge from stimulation of the anterior thalamus, as to whether patients with acquired lesions in such an area could in fact present as "petit mal" epilepsy. However, reports in the literature have been found of tumors in this area which are accompanied by suggestive patterns reminescent of those seen in"petit mal epilepsy." Dr. Ajmone Marsan and Dr. William Lewis present the clinical-pathological and electrographic findings on two patients in whom the electroencephalogram demonstrated an almost typical or highly suggestive pattern of "petit mal epilepsy." One such patient had a large metastasis of one cerebellar hemisphere pressing upon the brainstem, and the other had "petit mal seizures" and generalized convulsions together with a large astrocytoma in the right temporal lobe extending toward the thalamus. They then reviewed all similar cases of brain tumors in the literature associated with the bilateral 3/sec. wave-and-spike complex. Such cases are so rare that the question of a coincidental association of two unrelated phenomena could well be raised. These investigators therefore undertook a statistical analysis which would show that if chance alone were operating, such an association would be 48.2 of 1,000,000 cases. Thus, in the three published series totalling 1,071 brain tumors, with EEG correlation, the expected frequency of 3/sec. spike-and-wave would be that of 0.0517 case, but the actually obtained frequency was six cases. This discrepancy is highly significant and the authors feel that this is evidence that the two phenomena are indeed related and not coincidentally present.

The Branch of Electorencephalography has also carried out an electroencephalographic analysis of metastatic tumors of the

brain. Ninty-three patients with intracranial metastases were utilized in this study, all of which were verified at autopsy. In the case of intercerebral lesions, a high degree of correlation was found between the EEG and neurological findings. A few neurologically silent metastases were detected by EEG and, conversely, a few with positive neurological signs were missed by the EEG. By using strict and uniform electrographic criteria, the most significant factor appeared to be the absolute size of the metastatic lesion. A diameter of 2 cm. represented the borderline size for a lesion which would be detected by both neurologic and EEG examination (presence of focal delta activity).

Dr. John Van Buren and Dr. Ajmone Marsan have continued their data collection on patients with chronically implanted electrodes. The numerous records from nine patients are now being analyzed, 14 to 42 electrodes having been chronically implanted in each patient. Such electrodes have been placed within the thalamus, the basal ganglia, and in close proximity to either of these structures as well as subdurally. Such placements permit a direct, simultaneous recording of the electrical activity and a correlation of the superficial electrodes to those of different levels of depth. In addition to this, a technique of local stimulation with brief electrical impulses while recording the evoked potentials has produced promising preliminary results as to the preferential functional inter-relationship between various structures in the human brain. Correlation of the depth electrodes to the subdural electrodes appears likely to yield interesting findings which might be of use indirectly in routine EEG diagnosis as well as clarifying the role of such deep nuclei to both spontaneous and epileptic activity of the cortex.

Dr. Arturo Morillo and Dr. Ajmone Marsan are continuing their studies on the cortical control of sensory inputs. In such experiments, "cerveau isole" cats are used. Tungsten microelectrodes are stereotaxically oriented within the brain of the cat to record unitary potentials in the dorsal lateral geniculate nucleus. These were activated by light and/or by electrical stimulus of the optic tract. The elicitable response in the ipsilateral visual cortex by callosal impulses generated by electrical stimulation of the contralateral cortex was used as a "physiological" conditioning stimulus. These investigators found evoked potentials at the level of the lateral geniculate nucleus following stimulation of the contralateral visual cortex. Such efferent effects (i.e., cortical geniculate), are very likely related to the callosal response and consist of (1) activation of single unitary elements of the lateral geniculate body or (2) modification of the pattern of their firing when the latter is elicited by test photic or optic tract stimulation. Such a conditioning influence by callosal pathways could be inhibitory or facilitatory and could be shown for relatively short as well as

for fairly long intervals between the conditioning and test reflex suggesting both multisynaptic and shorter pathways. In view of this specific experimental design these investigators have convincingly demonstrated that such effects are not dependent upon anti-dromic phenomena and that there are indeed cortical-geniculate fibers. They now intend to use callosal responses in the visual cortex of the cat to study the morphology of the "slow" electrical potentials evoked in the visual cortex by stimulation of the contralateral homologous visual cortex. Combined with such surface recordings from large electrodes, these investigators will study the temporal distribution of unit activity similarly elicited and the relationship of such to the gross surface response. Such inter-relationships between the gross and unitary callosal phenomena and between the visual response elicited in the same cortical area by photic or optic tract stimulation will be studied in addition. At the present time this study is in the formative stage.

OPHTHALMOLOGY BRANCH

The Ophthalmology Branch reports on thirty-four separate projects reflecting an integrated program of basic and clinical work. Such studies include basic and applied approaches to visual processes, studies of intraocular pressure and its abnormalities, studies of refraction abnormalities, effects of radiation and pharmacological agents on the lens of the eye, the various abnormalities of the uveal tract, and on the neoplasias of the eye. For such studies, 151 patients were admitted for 8,297 patient days with an average stay per patient of 54.9 days. Once again, the integration of the various units of this Branch representing their varied disciplines highlights this report. Dr. Michelangelo Fuortes, Chief of the Section on Ophthalmology Physiology has returned to Italy to undertake the formation of a Department of Neurophysiology at Milan. His loss will be keenly felt within the Institute. Two Senior Visiting Scientists contributed much to the understanding of visual processes at the retinal level. These were Dr. Katherine Tansley, of the Institute of Ophthalmology in London and Dr. William A. Rushton from Cambridge University. Dr. Peter Gouras has returned to the Branch as a permanent senior investigator.

BASIC AND APPLIED VISUAL PHYSIOLOGY OF RETINAL ELEMENTS

Dr. Fuortes continued his studies before his departure utilizing microelectrodes for the intracellular recording of retina elements of the Limulus. In condition of dark adaptation, Dr. Fuortes found depolarizing transients from such cells following the application of weak lights (of the order of 10^4 quanta per second). A statistical analysis of the relationship between the frequency of appearance of such transient potentials and light intensity was performed by Dr. Fuortes, and the results

obtained were consistent with the hypothesis that absorption of one quantum may be sufficient for the generation of one transient. Another alternative, however, could be that an equal amount of light was sufficient for the release of a given package of chemical transmitter. In a study of the time course of dark adaptation, Dr. Fuortes used both light stimuli and depolarizing pulses directly through the microelectrodes. He found that the response to the depolarizing current recovered within a few seconds from the termination of the impulse but that the response from the testing light pulses recovered but slowly. He then studied the responses to such light and current pulses before, during, and after application of background illuminations of moderate intensity. He found that background illumination increased the response to currents but decreased the response to light. After application of a strong light which is then suddenly decreased to a lower intensity, the nerve cell recovers a large membrane potential but later depolarizes progressively to a steady value. A strong discharge of impulses which was present during the strong illumination is first abolished but later starts again at moderate intensity. Intracellular measurements indicated that the electrical conductance is decreased during the transient hyperpolarizing phase. If light is increased progressively so that the logarithm of light intensity is a linear function of time, a transient depolarization may still occur when a certain light intensity is reached (probably true only with gradual changes). The light intensity at which such a response becomes non-linear is not greatly affected by the rate of change of illumination. Whereas light will always evoke depolarization in the normal cell, if the cell is hyperpolarized by means of a steady current the effect of light may be reversed, i.e., become more hyperpolarized. This finding is relatively new when compared to other excitable cells and it is hoped that further studies may give more insight into such a mechanism.

Dr. W. A. H. Rushton brought to the Institute his elegant apparatus for the measurement of regeneration of rhodopsin in the human eye. The method consists of measuring the difference in the amount of light reflected back through the pupil from the fundus oculi before and after bleaching rhodopsin. The technique employs two beams of light traveling identical optical paths into the eye but oppositely polarized. One beam (516 mμ) is maximally absorbed by rhodopsin and the other (deep red) is not absorbed. When an analyzer is moved in their common path, the total light transmitted at any time can be represented by $r \sin^2 nt$ and $g \cos^2 nt$ where r and g are the intensities of the red and green beams respectively. Suitable adjustment of the intensity of one beam can make the output as well as the light reflected from the fundus constant over time. A change in the concentration of rhodopsin after a bright bleaching light will disturb the balance which can be regained by readjustment of the neutral wedge, representing a double density change in retinal

rhodopsin. The light reflected is measured by a photomultiplier, this output sent through a phase sensitive rectifier which compares it to another polarized signal changing at an identical rate; integrates the multiplied product (which is zero for all signals not changing at the rate the analyzer rotates and considered to be noise) and the final signal is measured by a galvanometer. Dark adaptation is measured by Gunkel's modification of the Goldmann-Weeker's adaptometer.

Using this apparatus, Dr. Rushton found that there appeared to be a critical point of 90% rhodopsin regeneration at which the rods begin to make their contribution to the threshold for light detection of the so-called rod-cone "kinck" in the dark adaptation curve. Since both rod curves of dark adaptation obtained by the different bleaching lights are superposable by lateral displacement along the time and since rhodopsin regeneration curves are likewise superposable on themselves, the coincidence between rhodopsin concentration and rod threshold demonstrable for only the final 10% of rhodopsin regeneration will therefore coincide through the entire regeneration curve. This is additionally supported by the argument from the kinetics of this reaction. The regeneration of rhodopsin is estimated to follow the rate of a first order reaction indicating that the retinene supply is maintained relatively constant during the process. Since the final 10% of regeneration occurs where the demands on retinene are the least, then the curve should be most accurately extrapolatable at this point.

Dr. Rushton comes to the conclusion that there is a fixed relation between the scotopic thresholds and the fractionation of opsin uncombined with retinene and that this relation is mathematically defined by combining psychophysical functions on dark adaption obtained from others and represents, hence, rhodopsin regeneration. The expression is $I/I_D=ay/y_o$ which shows that the logarithm of threshold is directly related to the fraction of opsin (y) uncombined with retinene; and therefore, rhodopsin concentration.

Using such an apparatus, Dr. Rushton then measured rhodopsin and dark adaptation in a subject with deficient cone vision (photanope). Dr. Rushton concluded that a photanope had about the normal amount of rhodopsin in the retina at 12° temporal to the fovea and that it regenerated at the normal rate. Any attempts made to measure visual pigments in the photanope fovea were marred, however, by irregular fixation, and within 2° of the centrally fixated field neither rhodopsin nor cone pigment could be detected.

In dark adaptation studies, however, it was demonstrated that in contrast to the normal subject, the photanope was not able to distinguish between yellow (580 mu) and green (520 mu) and presented a smooth rod curve. The cone threshold, however,

had a castellated appearance, as these photoreceptors were three times more sensitive to yellow than to green. No Stiles-Crawford effect was detected in the rod curve, whereas such an effect, although of low magnitude was demonstrated in the cone curve.

The situation in a rod monochromat permitted the plotting of the rod dark adaptation curve also above the level of the cone threshold. It could be determined, therefore, whether there is an inhibitory effect of rods by cones. In comparing the dark adaptation curve of the normal with that of the photanope, there was no indication of an inhibition of the rods by the cones, but suggested that the two visual receptors follow the independent mechanisms.

In a study dealing with the relation between rhodopsin and threshold in this patient, it was confirmed that the log $1/1_o$ in dark adaptation is proportional to the fraction of opsin uncombined with retinine. It does not, however, necessarily throw doubt on the tenability of Wald's compartment hypothesis of light adaptation as suggested by Dr. Rushton.

Along with Dr. M. G. F. Fuortes, Dr. Rushton also investigated the increment thresholds in a subject deficient in cone vision. He found such a subject apparently to have normal rod function and the rod increment threshold curve in the parafovea agreed with the results reported by other investigators as rod saturation was reached at 1,000 trolands. At this high illumination, increment perception was abolished and the patient lost the sense for visual contrasts.

Cone vision was restricted to the neighborhood of the fovea. The spectral sensitivity at this site was about normal and there was a Stiles-Crawford effect. The cone organization was not normal, however, because the threshold was extremely high, color vision almost absent, and visual acuity of the patient was low. On the basis of these observations, the existence of a cone-rod inhibition in the normal was questioned by these investigators.

As indicated in the previous Annual Report, the finding that diurnal squirrels have a pure cone retina which is maximally sensitive to the shorter wave-lengths, made these animals ideal for the study of spectral sensitivity curves. It was in the use of such animals that Dr. Katherine Tansley devoted her primary efforts aided by Dr. Richard M. Copenhaver and Dr. Ralph D. Gunkel during her stay at the Institute.

Electroretinograms were recorded from these squirrels in response to a light stimulus provided by a Xenon arc lamp and double interference filters. The experiments were always

conducted under both light and dark adapted conditions, and two stimuli tested through the whole spectral range. Sensitivity curves were plotted using the electroretinograms as the criteria. The retina of all seven species of squirrels examined showed the characteristics of a cone retina. The sensitivity curves were all found to be double humped, with maxima at 535 and 490 mu. While such humps were occasionally the same height more often than not, one or the other was markedly higher. A statistical analysis of the electrophysiological data revealed a mean hump or curve combining all the species examined. This curve was usually symmetrical with the two humps of equal magnitude. In addition, she noted a small but distinct reflexion between 450 and 460 mu. When the eye was adapted to color, the general sensitivity of the retina was depressed, but the results were calculated as the percentage of the maxima and it was found that blue adaptation depressed the hump at 490-500 mu while green to orange depressed it at 535 mu. The depression of the blue peak by blue light and the green peak by green light suggested the existence of two photosensitive mechanisms of different spectral sensitivities.

In view of recent work on photosensitive pigments (Dartnall 1960), Dr. Tansley postulated that the 535 mu peak is the result of interaction of some type between two photosensitive substances with an absorption maxima at 502 and 480 mu and that the 480 pigment is responsible for the peak in this region. If the two pigments were formed one from the other, as it is believed, this would explain the apparent rivalry of the mechanisms responsible for the two peaks, since the more of one pigment present, the less of the other. The off response of the electroretinogram was studied by Dr. Tansley since in the pure cone retina, a marked off-effect is a characteristic feature. She found that the intensity-response curves for the various parts of the squirrel electroretinogram showed that areas with increasing high intensity stimuli, (i.e., the a-wave and b-waves, increased in amplitude). The off-effect was then often depressed. During the development of the normal off-effect, a second superimposed a-wave was much enhanced while a second superimposed b-wave was depressed. The a-wave would return to its normal value about 100 microseconds after the off-effect while the b-wave took 200 microseconds when the off-effect was depressed by a strong stimulus, enhancement of the second a-wave was much reduced and sometimes delayed. The reactivation of the second b-wave was very much delayed. This strongly supports Granit's original supposition as to the nature of the off-effect in post-excitatory inhibition.

During dark adaptation, the off-effect of the red squirrel electroretinogram decreases in amplitude whereas in ground squirrels it increases. Flicker fusion frequencies for the red squirrel is considerably lower than in other squirrels. Histological examination of the red squirrel retina shows it to have

a less complex inner nuclear layer and fewer ganglion cells. Therefore, Dr. Tansley suggested that faster responses of the ground squirrel which resulted in a high flicker fusion are due to more efficient production of inhibitions and that this is associated with the remarkable development of the inner nuclear layer in this species. She suggests that interaction of P-III and P-II may be more important for the development of the off-effect in the red squirrel while in the ground squirrel the action of P-II of the off-effect may play a great part.

Dr. Sjoerd L. Bonting has turned his primary interests to the study of the biochemical, morphological, and functional events in the developing visual receptor cells, and used as his model, the DBA mouse from birth to maturity.

His objective was to establish the development and distribution of enzymes in the retina and particularly retinene reductase and retinene isomerase in the eyes of the same animals from birth to maturity, and to attempt to correlate whatever findings might occur with the morphological development of the visual cell as studied by light microscopy and electron microscopy, as well as with electroretinography in collaboration with Dr. Peter Gouras, and finally, to investigate such items for C3H mice and rats with hereditary blindness, as well as with rats treated with iodoacetate and glutamate. To do this, the Lowry-Bessey microcuvette had to be used and tissues weighed on the Lowry balance. In a normal albino rat, rhodopsin appeared at about the seventh day and the concentration increased rapidly and almost linearly to the twenty-first day and then became constant until maturity. The absolute amount of rhodopsin continued to increase, but after the twenty-first day, at a slower rate. The rhodopsin concentration and lengths of the whole rod as well as the outer segments were plotted against age. From the zero to the fourteenth day, there was a striking parallelism between the rhodopsin concentration and the outer segment length. The increase of the latter, however, becomes very slow after the fourteenth day, at which age rhodopsin concentration has reached only half of its maximal value. The electroretinogram in these rats first appeared at the tenth day, after which the amplitude increased rapidly and almost linearly until the twenty-first day. After this it, in turn, remained about constant.

In C3H mice which became blind due to hereditary degeneration of the rods between the 10th and 16th day after birth, a normal development of rhodopsin until the 10th day is found. Then the rhodopsin concentration begins to decrease and reaches virtually zero between the 16th and 19th day. In rats receiving injections of 3 X 20 mg/kg. iodoacetate over a 30 hour period in the eighth week of life, the rhodopsin will decrease by 40% without morphological changes being visible in the light microscope.

Dr. Bonting has initiated his preliminary studies on the enzyme retinene reductase from the rat and from the cow. The histochemical staining technique with tetrazolium was found to be unsuitable because it indicated "diaphorase" activity rather than retinene reductase activity. Chemical assays in retinal homogenates with ethylalcohol as a substrate following either DPN reduction or oxidation spectrophotometrically were unsuccessful thus far because of low activities and the occurrence of non-specific and even non-enzymatic oxidation-reductions. The parallelism between rhodopsin concentration and the electroretinogram is an important finding and is in agreement with the recent work by Dowling and Wald on vitamin A-deficient rats.

Similar toxicological studies were carried out by Dr. Ottiwell Jones on patients receiving the monoamine oxidase inhibitor, JB 516. Patients were receiving this drug for hypertension. Such patients were periodically studied for visual acuity, and biomicroscopic, funduscopic, and visual field examination, and color testing were carried out. Autopsy tissue was available from one such patient. Of the eight patients studied, all eight developed a decreased visual acuity and a red-green color defect. Two patients developed central scotomata in addition. Six patients recovered after cessation of the drug. The two patients with the central scotomata did not recover normal vision and one of the two patients died and lesions were found in each optic tract just anterior to the lateral geniculate bodies. This finding is of interest in relation to Dr. Bushnell Smith's investigations (see Medical Neurology Branch) and color vision testing in centrencephalic patients will be initiated.

Dr. Peter Gouras is continuing his electrophysiological studies of the retina of the vertebrate using amphibians in which he is recording with both intra- and extra-cellular microelectrode responses of the ganglion cell layer of the retina. The initial objective of this study is to determine dependency of the ganglion cell discharges on the rate of change of photic stimuli. Stimulus presentation is the unique part of this study and it has varied rate dependencies having slow, continuous changes obtainable by rotating analyzers in polarized light by using sinusoidal wedges or glow modulator tubes. Dr. Gouras believes the ganglion cells of the amphibian retinae have the properties of "differentiators". By this, he means that any photic stimulus can be substituted for any other differing in chromaticity without the ganglion cells detecting the change if the substitution is slow enough. He found that ganglion cells which discharge to a flickering stimulus varying as the function $\cos^2$ wt. (wt is the rotation of a polariod in radians/second) trace different curves to different wave lengths when the flicker fusion frequency is plotted against intensity. Long wave length stimuli produce steeper curves than short wave lengths suggesting that the rod function (scotopic) and the cone function (photopic) mechanisms

interact on a single ganglion cell; that the long wave lengths are more effective in determining critical flicker fusion at high intensities and the short waves, critical flicker fusion at low intensities in a manner analogous to the Purkinje shift. A surprising result obtained was at either low intensities or low flicker rates the curve may turn back on itself so that fusion results. Thus, fusion was obtained not only when the stimulus was too fast but also when it was too slow.

To clarify signal to noise ratio in determination of the electroretinograms, Dr. Gouras has turned to electronic computers. At the present time, he has a study under way which includes ten patients with pigmentary degeneration, all of whom had "extinguished" electroretinograms on the conventional recording system. All patients, however, had responses, including some with only 5° central fields. He found a direct relationship between the functioning retina as estimated by perimetry and the amount of light necessary producing a constant amplitude electroretinogram. Spectral sensitivity curves determined on such patients by this technique revealed a predominantly photopic curve in patients with functioning macular retinae and scotopic in those with more rod vision. This relationship between the retinal area and the electroretinogram threshold in retinitis pigmentosa, Dr. Gouras feels weakens the Bruch's membrane short circuiting theory proposed to explain the absent electrical response in retinitis pigmentosa patients with only moderate retinal pathology. In addition, Dr. Gouras has been recording the occipital evoked response during each such study. He has demonstrated a depression of the evoked response in a subject with migranious scotoma. Dr. Gouras is also investigating the electroretinograms in animals used in the study and development of rhodopsin by Dr. Bonting.

Finally, Dr. O. W. Jones and Dr. Ralph Gunkel are carrying on functional studies in retinal anomalies and diseases using electroretinography, adaptometry, and perimetric light sense studies. This is a continuation of the study of the previous year utilizing the Goldmann adaptometer and the "retinal profile," on Dr. Gunkel's modification of the adaptometer. Electroretinography was performed on every patient utilizing the contact lens electrode and the Grass 8-channel electroencephalograph. This is largely a correlative study and could be compared to the electromyographic studies now undertaken in the Branch of Medical Neurology. Here again, the results of such studies made it possible to give the correct diagnosis and prognosis in the majority of such cases. A few cases of achromatopsia were examined and significant electroretinographic data were obtained to correlate with the bleaching and regeneration experiments done by Dr. Rushton.

GLAUCOMA AND MECHANISMS OF AQUEOUS FORMATION

Doctors Sjoerd Bonting and Kenneth A. Simon are undertaking a study of membrane ATP-ase and aqueous formation. Following the studies of Skou, who demonstrated in the crab nerve that this enzyme is closely connected with sodium and potassium transport across membrane, these investigators found ATP-ase activity in the following tissues of the cat: cerebral white matter, gray matter, the optic nerve, the choroid plexus, ciliary body, kidney cortex, medulla and papilla. Such ATP-ase activity could be inhibited by the omission of the potassium ion or the addition of calcium ion or ouabain. Ouabain concentration at which 50% inhibition occurred in the ciliary body was found to be 5×10^{-7}M, which is close to that previously reported for the erythrocyte membrane ATP-ase. Diamox in 10^{-4}M concentration fully inhibited the membrane ATP-ase of the ciliary body and choroid plexus. These findings suggest that ATP-ase may play an important role in aqueous formation.

Dr. Frank Macri is continuing his studies on various pharmacological agents affecting the intraocular pressure and their relationship to the venous pressure and arterial pressure within the eye. He has found that epinephrine and arterenol in concentrations of 10^{-12} produce a rise of iris artery pressure and a slight decrease of perfusate flow. When these agents are utilized in doses of 10^{-6}, only a fall in iris artery pressure flow was greatly reduced. Doses between the two generally produced a biphasic response beginning with a rise in the intra-arterial pressure and then proceeded to fall below the control levels. He proposes the following mechanism for such agents: at low doses there is a constriction of the radial arteries of the iris and at high doses, a constriction also of the iris artery. In preliminary studies, Dr. Macri found that low doses of acetylcholine at a concentration of 10^{-12} lowered the iris artery pressure and increased the perfusate flow. He feels that this action is explainable on the basis of dilatation of the radial arteries of the iris. In studies with Diamox, doses of 5 mg/liter which is approximately equal to the human dose, he found that this drug produced a rise of iris artery pressure and a decrease of perfusate flow. This effect is compatible with a constriction of the radial arteries of the iris.

In previous studies on Diamox, the thesis was presented that carbonic anhydrase inhibitors and the sympathomimetic amines reduced the intraocular pressure by diminishing the secretion of aqueous humor. In the present studies, however, apparently at least part of the lowering of intraocular pressure induced by such agents, could be ascribed to a constriction of the iris arteries. Therefore, Dr. Macri turned his primary attention to the role of the vasculature in maintenance of intraocular pressure.

He measured the intraocular pressure and the vascular pressure in the various parts of the eye and body. Correlations were made between the intraocular pressure and vascular pressures to show the relative dependence or independence of these various systems.

In cat, he found by simultaneous measurements, that the pressures in the anterior ciliary vein and the vortex veins were identical. He felt this to be explained by the anastomotic communications between the two systems as demonstrated by the cast method discussed in the previous report. In a series of 39 cats, simultaneous measurement of the normal intraocular pressure and "head-on" venous pressure was made at a time when the two variables had been constant for at least 15 minutes. The intraocular pressure was then plotted as a function of the venous pressure; the regression was linear and highly significant and could be expressed by the equation, the IOP= 0.82 VP + 6.51. In such experiments, the measurement of the venous pressure necessitated a total occlusion of the vein and cast, therefore some doubt on the validity of the findings. To meet such criticisms, Dr. Macri reinvestigated this relationship by the use of microcannulae inserted through the wall of the vein upstream so that the vessel remained patent. Again, a highly significant regression was obtained which was almost identical and could be expressed by the same equation. That the total occlusion of one vein in the first series of experiments had no obvious effect on the IOP/VP relationship but produced a mean rise of 20 mm. of mercury in the intraocular pressure was suggestive evidence that the venous pressure may be a dominant factor in the maintenance of intraocular pressure.

To study this further, another series of experiments were undertaken. Using intact eyesof anesthetized cats, the venous pressure again was measured "head-on." Acute changes of blood pressure were produced by the administration of various agents which in turn produced acute changes in both intraocular pressure and venous pressure. They were plotted against each other and the curves of 12 such experiments were grouped and could be expressed by the equation now IOP = 0.90 VP + 3.42. Using enucleated and arterially perfused eyes, a series of 45 eyes were examined. Again, a highly significant linear regression was found between the intraocular pressure and the "head-on" venous pressure which could be expressed by the formula IOP = 0.77 VP + 7.43. Finally, a series of eleven eyes were perfused as above but the intraocular pressure and venous pressure were varied in each eye by raising and lowering the perfusion pressure. The mean equation obtained at this time was IOP = 0.89 VP + 3.58. Therefore, it became apparent that the different procedures used to produce a primary effect on the vasculature of the eye all produced a relationship of IOP/VP similar to that found in "normal eyes." When aqueous humor volume was changed, it was found that IOP/VP relationship could be ascribed by the

equation IOP = 1.74 VP - 18.87. This equation varied significantly from those obtained in the 39 cat series.

Dr. Macri concluded that since the changes of venous pressure produced values of "K" in relationship IOP = K VP + C similar to that obtained in "normal" eyes and mice. Changing the volume of aqueous humor resulted in significantly different "K" values; the intraocular pressure of the eye is dependent in a large part on the venous pressure.

Since no relationship had been found at steady states between intraocular pressure and systemic blood pressure by these experiments and accepted clinical experience, yet it is well-known that the acute changes of blood pressure produce parallel changes of eye pressure; Dr. Macri postulated that some slowly compensating mechanism existed to explain the lack of correlation of the intraocular with the systemic blood pressure at steady states. The two most likely possibilities he felt for this would be alteration of aqueous humor dynamics, i.e., the outflow and inflow, or vascular pressure dissociations occurring somewhere between the heart and the exit veins of the eye. This latter possibility was explored. Correlation measurements between the systemic blood pressure and ophthalmic artery pressures were made specifically between pressures of the ophthalmic artery and iris artery and between the iris artery and intraocular pressures. It was thought that a dissociation of pressure within any of such pairs might indicate the control of one vessel of such a pair. Dr. Macri found a very high correlation between the femoral artery pressure and the ophthalmic artery pressure and concluded that the ophthalmic artery pressure varies continuously with the general blood pressure of the animal. No correlation, however, could be found between the pressures of the ophthalmic and the iris artery under the conditions of the experiment. In contrast to this, a highly significant correlation was obtained between the iris artery and the intraocular pressure.

He concluded, therefore, that the pressure in the iris artery is not associated with general blood pressure and dissociation occurs at the level between the iris artery and the ophthalmic artery. The mechanism of such dissociation at this time is unknown. The equation relating the intraocular pressure to the iris artery pressure was IOP = 0.82. IAP - 4.90. Since the slopes of this equation and the equation of intraocular pressure to venous pressure are the same, he concludes that there is a 1:1 relationship between the venous pressure and iris artery pressure. Dr. Macri now plans to see if similar correlations hold for higher mammalian species.

Dr. Scullica with Dr. Macri studied the flow pattern in the ciliary process of albino rabbits. In albino rabbits, the ciliary processes extend upon the posterior surface of the iris

and go beyond the circulus arteriosus. Because of the lack of pigment, the blood vessels of the ciliary processes are visible and the flow of red cells can be easily observed and studied. Eyes were, therefore, enucleated and the artery cannulated with a hyperdermic needle and a solution of Krebs-Ringer solution, horse serum, and red blood cells were perfused through the arterial system. These investigators found the blood supply of such processes to be composed of a variable number of vessels which have their origin from the arteries of the iris and the ciliary body. Such vessels entered the base of the process at various points and branched either into a larger number of small vessels or into a small number of larger vessels. On the dorsal portion of the ciliary processes was one large vessel which began at the tip to join the choroid. At the beginning of the arterial perfusion, one can see the red cells coming from the radial branches of the iris artery into the anteriormost artery of the process and then proceed from there to the large vessel at the dorsum and hence to the choroid. Immediately afterwards in the majority of such eyes, the flow was observed in other arterioles and capillaries which goes to the same dorsal vessel. The dorsal vessel has the characteristic of a vein and has been called by these investigators the marginal or dorsal vein. Occasionally, the anterior portion of the processes is shunted from the flow and in these cases, the main circulation of the blood cells proceed through an arterial vessel which goes to the middle or posterior part of the ciliary processes and to an ancillary dilated capillary bed which then again communicates directly with the marginal vein. This marginal vein at the level of the orbiculus, divided into two branches, one superiorly, and one inferiorly. The superior one then went on into a capillary net which lay between the choroidal and iridiociliary vessels. Inferior vessels proceed underneath the ciliary processes and joins the choroidal veins. Thus, the flow can be seen to proceed from the marginal vein to either or both of these vessels. The anterior portion of the ciliary processes had a second mechanism of flow, which was but rarely seen, made up of a large vessel which originated from the capillaries and proceeded under the processes to the choroidal vessels.

Dr. Edward Okun and Dr. Ludwig von Sallmann have continued their clinical glaucoma study in an attempt to evaluate suspected known cases of glaucoma and to find better means of diagnosis and control, as well as to further investigate the disturbed intraocular fluid dynamics in glaucoma. Just as tonography gives an indication of the ease with which fluid leaves the eyes, the inflow studies give an indication of the rate which the fluid entered the eye; 1,794 patient days were utilized in this study. Extensive glaucoma workups were performed on each patient which include tonometry, applanation tonometry, biomicroscopy, gonioscopy, and repeated tonography with and without provocative tests. In addition, the visual fields were carefully plotted using the Goldmann perimeter and the Gunkel tangent

screen. This is still in the initial phase and reliable studies are as yet not completed. It appears, however, even initially that tonography when properly performed has proven to be of great value in conjunction with dilatation provocative testing for narrow angle glaucoma. Studies are underway at the present time in cases of borderline glaucoma for the positive water drinking provocative tests. Applanation tonometry, as seen in these initial studies, may be fairly sensitive means of measuring inflow.

THE CORNEAL ENDOTHELIUM

Enzymatic zonulolysis is now widely used for cataract extraction. During the surgical procedure, injuries to the endothelium of the cornea occur. An investigation was initiated by Dr. Huckel to see whether alpha chymotrypsin interfered with the endothelial wound healing. The number of cells undergoing mitosis is taken as an indication of such an effect. When animals were killed 24 hours after the injury to the cornea, the number of dividing cells were slightly less in the eye treated with alpha chymotrypsin. This difference disappeared at a 48 hour interval. Local instillation of l-epinephrine also depressed mitotic activity. This investigator concluded that in young, healthy rabbits the depressant effect of chymotrypsin on cell division is of low magnitude and transient in nature. The use of this enzyme in cataract surgery cannot be considered as a hazard by delaying endothelial regeneration.

Dr. Ludwig von Sallmann has continued his investigation as to the soundness of the generally held concept that mitosis does not take place in the endothelium of the cornea. Using his elegant technique of flat mounts which are required to contain the whole cell population, corneae were processed with the Feulgen reaction and mitotic counts obtained from both eyes of more than 60 animals. In contrast to the generally held opinion that the mitotic division in the endothelium is present only shortly after birth, Dr. von Sallmann found that the mitotic activity actually exists in the endothelium of growing animals. The dividing cells were more numerous in the peripheral zone of the cornea than in the center and that their number decreases with age. The mitotic index was calculated by counting the cell nuclei per microscopic field and taking planimetric measurements of the whole area of the mount. Such mitotic activity, however, could not explain the increase of the size of the cornea by cell division alone and increase in cell size and flattening of the cell body greatly contribute to the growth process. Diurnal variations were demonstrated by early morning and afternoon counts. The ratio of these two counts increased with age. Dr. von Sallmann concluded that after growth had stopped, the corneal endothelium behaves as a non-renewal area where dividing cells in fully grown animals are few or missing. Such findings places the corneal endothelium in the group of tissues of which the vascular

endothelium and the endothelium of the peritoneal cavity are also examples.

In his studies on incorporation of tritiated thymidine in the corneal endothelium of the rabbits, Dr. von Sallmann used the osmotic pump to allow chronic administration of chemical agents into the anterior chamber at a very slow rate of speed, i.e., 0.5 ml/24 hours, by using fine polyethylene tubes, measuring from 100-300 microns in diameter. This study is in a preliminary stage, but it has been shown that the number of labelled nuclei seemed to exceed the number of cells which were calculated to pass through mitosis during a day; and that the labelled nuclei were abundant in areas which appeared to have been injured but the incorporation of the isotope was also seen a great distance from such an injury. In the normal endothelium, labelling seems also to take place in the central part of the cornea where dividing cells are usually thought to be absent as noted in the previous study.

STRUCTURE, PHYSIOLOGY, AND ABNORMALITIES OF THE LENS

Dr. Robert Resnik is continuing his studies on the proteins of the lens, in particular, alpha F and alpha G. In his studies of the physical constituents of crystalline of the lens, Dr. Resnik has found that Alpha F and Alpha G crystalline transform into particles of lower molecular weight. This is indicated by the appearance of slower sedimenting material in the ultracentrifuge patterns. The molecular weight of the native and fully transformed alpha G crystalline as determined by the approach to equilibrium methods in the ultracentrifuge are 1,086,000 for the native variety, and 514,000 for the transformed variety. The sedimentation coefficient of 0.29% solution of alpha G crystalline in 0.05 M phosphate buffer at pH 6.8 is 20.1×10^{-13}. The diffusion coefficient of the same sample is 2.0×10^{-7}. These values will give a molecular weight then of 940,000. A 0.41 solution of fully transformed alpha G crystalline gives sedimentation and diffusion values of 14.6×10^{-13} and 2.7×10^{-7} respectively. These parameters give a calculated molecular weight of 509,000. Such a pH induced decrease in the molecular weight of alpha G crystalline is irreversible. Dr. Resnik feels this obviates Bon's hypothesis of a change in shape without a change in size. The charge of the molecule of pH 8.7 does not appear to be altered since boundary electrophoresis does not reveal the two forms of alpha G crystalline when known mixtures were used.

Dr. Theodor Wanko is continuing an electron microscopic study on tissues of the eye, in particular, the lens epithelium and lens fibers after cataractogenic agents have been administered, as well as examination of human cataractous lenses obtained immediately after operation. He is also studying the

morphology of the ciliary epithelium with the electron microsope as well as the human conjunctiva. His studies on the lens are a continuation of last year's report on the suture areas in the cortex of the normal mature rat and rabbit lens. It is noted in the vicinity of the suture, the regular array of the lens fibers membranes gradually changes to a system of many protruding cell portions interposed and interdigitated between similar projections of the adjacent cells. In such areas of the lens, specialized contact areas of cell boundaries classified as desmosomes are present. Such interdigitations of cell surfaces may preserve the morphological integrity of the cell during the process of accommodation.

In experimental cataracts, Dr. Wanko examimed the myleran cataracts at 2,4, and 6 weeks, as well as the mimosine cataracts at 5, 7, and 14 days. He now has eight human senile cataract lenses. The normally homogenous lens capsule occasionally exhibits (in the senile cataract) an interposition of dense granular material in a regular arrangement. In the epithelium as well as in the cortical lens fibers, structures resembling fragmented or disintegrated cellular constituents may be seen.

Nine conjunctival specimens from Sjogren's syndrome have been obtained. Studies are now in progress on this. As noted above, Dr. Wanko's laboratory is also active in delineating the subcellular particles used in the DNA and RNA determinations of the Section of Neurochemistry and is participating in the elucidation of many neuromuscular disorders as noted in the Branch of Medical Neurology.

Dr. von Sallmann and Dr. Howard J. Curtis of the Brookhaven National Laboratory are studying the effects of high energy proton microbeams, simulating cosmic primaries on the mouse lens. It has been assumed that the biological effect of cosmic ray particles result from the dense cluster of secondary protons surrounding the cosmic ray alpha particle. Such a cluster is said to create a core of ionization about 25 mu in diameter. Beams of 25 microndiameter and larger were obtained with a 22.5 MEV deutron beam from the 60 in. cyclotron of the Brookhaven National Laboratory. The beam was directed through round apertures 25,75, and 1,000 microns placed in front of the target area. The beam was well collimated to a depth of 1.5 mm. Thus, the peak of the Bragg curve was expected to be found at a circumscribed site of the equatorial lens cortex. Doses administered were from 1,012 to 33,000 rads. The animals were studied biomicroscopically in bi-weekly intervals and killed at varying times for the histological examination. Cytological examination of flat mounts of the epithelium was also carried out in a small portion of the series which, at the present time, comprises the eyes of 130 mice. Thirty-two mice were set aside to study the late effects. Opacities were visible in the biomicroscope

resembling fine round dots or elongated irregular shapes in the middle superior sector of the posterior cortex in a small group of the mice exposed to the 25 micron beam of 33,000 rads, 20 weeks after radiation. The lesions did not seem to progress in the following months. Radiation with a 75 micron beam at a dose level of 5,200 to 13,000 rads produced signs of radiation injury much more frequently and at a shorter latency period. Lower doses caused minute changes five to six months after radiation. With the 1 mm. aperture, doses of 5200 rads produced fine opacities in 20-22 weeks. Subsequently, the number of these opacities increased and in several instances, were accompanied by a diffuse cortical opacification around the posterior pole. Similar signs of radiation damage were observed in doses from 1012 to 2600 rads. The histology of such radiated eyes did not always parallel the results of the biomicroscopic studies. In the group irradiated with the 1 mm. aperture, migrated and degenerated bow nuclei and swollen fibers were seen as early as four weeks after exposure to 4700 rads or more. At the 25 microbeam, such changes occurred at higher dosages, i.e., 13,000 or 33,000 at the 12 to 14 week interval. At six-month intervals, irradiation with 1012 rads through the 1 mm. aperture induced cataractous changes in all animals. The use of the lens as against other tissues is important in this study in that the lens is avascular. Thus, in similar studies on the brain, the Brookhaven Group came to the conclusion that the larger radiation area of the brain was dependent in part upon vascular disturbances. However, the lens being avascular removes the effects of such tissue damage. In the observation of mouse lens, it can be assumed that the biological effect of the simulated cosmic primaries are of a low order for this organ. The question of recovery from minimal radiogenic lesions in the lens and restoration of the lens to normal still needs further study.

The finding that 17 of 44, i.e., 39% of patients, with rheumatoid arthritis on prolonged corticosteroid therapy had posterior subcapsular cataracts and that 19 patients untreated with corticosteroids did not have these cataracts led Dr. von Sallmann and Dr. Oglesby to investigate this phenomena. They found that the occurrence of these cataracts was correlated with the dose and duration of the corticosteroid therapy and not with the age of the patient, the severity of the disease, or therapy received. Although approximately 50% of the patients receiving chronic corticosteroids have abnormal glucose tolerance curves, there was no concomitant finding that cataracts were present more frequently in those than with abnormal curves than those without. Patients with other collagen diseases treated with corticosteroids have also been observed to develop such cataracts. The ultimate outcome of the cataractous process is still undetermined until such patients have been followed for longer periods of time. One such patient has come to post-mortem, and in this patient, the primary finding was that of swelling of the lens fibers underneath the capsule and that no migration of nuclei were noted as

is seen in radiation cataracts described above. Thus, the radiation received by such patients with chronic rheumatoid disease does not appear to be, at least in this case, a cause of the cataract.

To elucidate this further, corticosteroids were given chronically to 35 male rats of the Sprague-Dawley strain, in which 15, 10, or 20 micrograms of Decadron were given to such rats. Control animals were injected similarly and food intake, weight gains, and so forth, were checked periodically. The major findings were a decreased weight gain of the differentially treated animals when compared with each other and controls, and this indicated the effectiveness of the steroid injections. The retardation of body growth was greatest in the animals receiving the highest dose of the corticosteroid. No biomicroscopic signs of cataract formation was found. The mitotic counts in the lens epithelium in the treated eyes were of the same magnitude as those of controls. The alleged depressant effect of steroid therapy on cell division could not be demonstrated on the lens epithelium. Skin abcesses reported to occur at the site of injection were not found and the suggestion that these complications caused the decrease of weight gain could therefore not be substantiated. It was concluded by these investigators that the chronic administration of high dosages of corticosteroids continued for four months does not affect the lens of the young growing rat.

REFRACTION ANOMALIES

In his summary of the Ophthalmology Branch, Dr. Ludwig von Sallmann has drawn special attention to the new conceptual approach of Dr. Gerald van Alphen to refraction anomalies. In this study which has now taken approximately three years, Dr. van Alphen by mathematical treatment of the data provided in the literature of correlation of optical elements, shows that the present theories (Steiger) as to the mechanisms operative in emmetropia and ametropia do not, in fact, provide an answer to the fundamental mechanisms at fault. Dr. van Alphen went on to show that the links between the optical elements could be best explained under the assumption that the choroid itself behaves as a sheath muscle, which is able to counteract in part, at least, the intraocular pressure. He then went on to measure the tension and tension changes of the choroid as well as the effectiveness of the elasticity of the sclera and the response of both to pharmacological agents. Parasympathetic stimulation results in contraction of the ciliary body-choroid unit. In experiments on isolated strips of this tissue, acetylcholine also brings about a contraction, the extent of which depends upon the tension of the preparation. Adrenergic compounds, however, produce a relaxation. The results of these experiments provide

experimental data to strengthen the statistical analysis of the correlation of the optical elements of the human eye, and they in addition leave the inference that the study of the relation between the muscle sheath and the pathology of intraocular pressures should also be undertaken.

UVEAL TRACT INFLAMMATIONS

One of the largest studies of the Ophthalmology Branch and one which has been followed almost since the conception of this Branch has been a study of ocular toxoplasmosis. A large number of patients have been continually referred to the National Institutes of Health with definitely active uveitis, a majority of which were resistant to previous therapy. Of such patients, 43% showed a complete disappearance of activity of the lesion after treatment with Daraprim (pyrimethamine) and sulfonamides.

Patients with uveitis responding best to such treatment, as pointed out in previous reports, have the disease before the age of twenty and the uveitis is unilateral and is not exclusively anterior. There was no correlation between the level of the dye test and the therapeutic responses.

Dr. von Sallmann and his colleagues feel that the toxoplasma skin test appears to be an excellent screening test for antitoxoplasma antibodies and was positive in 95% of patients with positive toxoplasma dye tests and who had not had steroid therapy immediately before skin testing. Only one patient of the 200 controls had a positive toxoplasma skin test and this was an atopic patient who responded to all antigens. In their recent series of patients, however, these investigators find that the toxoplasma skin test was negative in several instances in patients with a high toxoplasma dye test titer. Patients who do not tolerate therapy well and respond with a servere depression of the platelet count will be treated with Vitamin B-12 and folic acid.

Dr. Theodor Wanko is doing an electron microscopic study of the protozoon Toxoplasma gondii. Dr. Wanko points out that initially toxoplasma cysts were collected by a micromanipulatory procedures from homogenized infected mouse brain. Such isolated cysts studied in the initial phase of this project are providing some information as to the presence and gross arrangement of organisms inside the cell wall but this had to be abandoned since the isolation of the cysts from their natural environment prevented the simultaneous observation of the surrounding tissue. In the micromanipulation, only the largest cysts were selected and no information on cysts development could be expected. Therefore, Dr. Wanko turned to the brain tissue itself and demonstrated therein that the organisms were encapsulated by an opaque

layer within which vesicular and membranous elements could be resolved. Within the cyst itself, the space between the cell boundaries of an individual organism and its partner was filled with an homogeneous material which is in continuity with the capsule and is of similar opacity. The observations at present suggest that such opaque material between the organisms was derived from the organisms whereas the membrane and vesicular profiles in the cyst wall may correspond to remnants of the host cell. Individual organisms have been seen in brain capillaries and intracellularly in the brain.

The cellular organization of the organism in the cyst is characterized by the following constituents: nucleus with a nucleolus; mitochondria, Golgi complex, endoplasmic reticulum, toxonemata emanating from the conoid, a sub-membranous fibrillary apparatus, vacuoles, and a continuous cell membrane.

OCULAR TUMORS

Dr. James F. O'Rourke's study utilizing P^{32} with detection by a posterior global probe was finished as of the last year at which time it appeared that serious deficiences were present in this technique. Further studies in ocular tumors were undertaken this year by Dr. Bruce E. Cohan who reported on an apparent epidermoid carcinoma of the nasopharynx involving the lacrimal sac and presenting itself in a patient with the symptom of epiphora. The differential diagnosis between primary lacrimal sac carcinoma in nasopharyngeal epidermoid carcinoma was discussed at some length.

NEUROSURGERY BRANCH

The efforts of the Branch of Surgical Neurology were directed toward the further understanding of the normal physiology of superficial and deep nuclei of the human brain and the abnormalities of the same, associated with abnormal cerebral discharges, directed particularly to the temporal lobe. This study, as in previous years, was a joint effort involving primarily the neurosurgeon, the electroencephalographer , the radiologist, the pathologist, and the psychologist. This was accompanied by fundamental studies at a cellular level. The coordination of this team was under the direction of the Branch Chief. The branch in addition, has now launched a major program in the study of the basic mechanisms underlying involuntary movements, as well as surgical procedures towards the alleviation of the same. Detailed studies in the vascular permeability of cerebral vessels and cerebral edema have been carried out using immunohistological techniques. Studies on the functional anatomy and pathology of the human visual system have continued. The effects of deep hypothermia in higher primates, and radiofrequency wave effects in the brain of primates were investigated. Abnormalities detected on and about the perinatal period have been studied.

Toward this end, 234 inpatients have been admitted during the past year. Three hundred and nine visits to the Outpatient Department were recorded and there were 73 consultations. One hundred and eighteen major surgical procedures were done of which 41 were epileptics, 22 had intracerebral tumors, 20 suffered from involuntary movements for which stereotaxic procedures were performed and 35 operated on for miscellaneous lesions requiring surgical treatment. The branch prepared 23 reports for publication.

EPILEPSY AND FUNCTIONAL ANATOMY OF THE BRAIN

During the reporting year, under the supervision of the Branch Chief, 130 patients with cerebral seizures were studied in the operating room and on the ward. Of these, 70 were localized to one or both temporal lobes. Studies were concerned with behavior, language, and response to high and low sodium diets, and response to various anti-convulsants. Eleven of such patients were also studied by Dr. Van Buren with depth electrode techniques. The Branch Chief at this time summarizes the entire experience over the years by the Surgical Branch with focal seizures and notes that now over 300 such patients are recorded. One hundred and seventy-two temporal lobectomies were included in this group and the results of 88 such cases have been reviewed by Dr. Donald Maccubbin and the Branch Chief. Such cases were followed for periods of one to eight years. Coincidentally, with this study, Dr. Robert Savard of the Social Service Unit of the Clinical Center completed a social study of the postoperative effects in 33 cases now five years after operation. Of the 88 cases, 38% were seizure-free while 75% were considerably improved. Dr. Savard's study showed clearly an improvement in both family and community relationships as well as economic usefulness.

In a review of the surgical anatomy of the temporal lobe, Dr. Maitland Baldwin came to the conclusion that removal of the lateral cortex alone does not relieve the seizures but such a removal must include mesial structures, but paradoxically one can exclude the pes hippocampus. If this latter structure is removed then serious disturbances in recognition and recollection may follow. The excision of the amygdalae did not seem to produce discernible affects, but did seem necessary for the irradication of seizures in most cases.

The psychoses related to temporal lobe seizures were studied by Dr. Philip Ferris and Dr. Baldwin in 25 cases characterized by overt-psychotic episodes. The majority of such episodes consists of dissociation and bizarre language accompanied by hallucinations. These investigators came to the conclusion that persons with epileptic disease of the temporal lobe are prone to a schizophrenic-like psychoses and such a psychosis is an exaggeration of the inter-ictal behavior in all cases and

could be precipitated or exaggerated by withdrawal of anti-convulsants. They did not note such psychosis when other parts of the brain were involved in abnormal seizure patterns. The removal of the affected lobe would lead to relief of the psychotic episodes.

In cooperation with the nursing staff, under Mrs. Mary Thompson, over 6,000 spontaneous ictal seizures have now been recorded by photographic means. An exhibition of this study has been prepared for the American Medical Association.

The effects of high and low sodium diet on seizure frequency has also been studied and it would appear that electrolyte regulation in so far as can be done by diet, plays an important therapeutic role . In the laboratory, utilizing higher primates, (i.e., chimpanzees) the study of penicillin-induced lesions continues. Once a penicillin-induced seizure activity is maintained in an area approximately 1 cm. square on the cortex of the chimpanzee and monitored locally, the injection of 10% sodium chloride by vein, will cause the intermittent firing in such an area to become continuous and subsequently spread and the disturbance becomes diffuse. If such a lesion is allowed to become inactive so that the discrete focal spikes are no longer recorded, it may, in turn, be reactivated within 30 minutes after the intravenous injection of 10% sodium chloride. If the animal is made hypotensive, no change of any type is found in the firing of the lesion. This lesion is being studied as far as vascular permeability by Dr. Igor Klatzo and Dr. Richard Otenasek, by means of tagged-albumin and ordinary fluorescent techniques. Functional representation within the temporal lobe of man and higher primates has continued utilizing electrical stimulation and recording of the human, chimpanzee, and monkey temporal lobes during operative exposure, and indirectly by depth electrodes and scalp recordings. Ablation experiments of the same lobes in higher primates and anatomical studies of the whole brain after temporal lobe excisions are continuing. In addition to this during the past year, studies on the parietal cortex have been added. In one preparation, the parietal cortex has been ablated on both sides and paradoxically, no parietal syndrome was noted. This preparation will now have an ablation of the adjacent temporal cortex as there is some evidence that in a large amount of conjectures that the temporal and adjacent portions of the parietal cortex act together and that this large association area is, in fact, a complete functional unit. The removal of the dominant hemisphere in the chimpanzee does not in this study interfere with communication, however, in the case of 172 temporal lobe excisions in man, Dr. Baldwin and his colleagues indicated changes in affect, language; in some cases sexual function. It is no interest that Dr. Savard's studies seem to show that speech improves after right-sided temporal lobectomy. The Branch now reports some 532 temporal lobe stimulations in the human

and again are collecting secondary sensory responses. The number of so-called psychical responses from surface stimulation seemed to be a rare event. Nonetheless, there are 60 such responses in the series at this time. Motor responses have been found from the postcentral area as well as precentral. The relationship suspected between amygdaloid stimulation and autonomic responses have not been confirmed. This group now anticipates the publication of a monograph based in 172 cases of right or left temporal lobe excision and 532 responses to stimulation of temporal region. Social studies, operative results, electroencephalographic and electrocorticographic tracings, depth stimulation, and anatomical connections, as well as Dr. Dekaban's data on the embryological development will also be included. Once again, the utilization of a wide variety of hallucinagens has demonstrated in the chimpanzee whose temporal lobes have been removed, that no responses to such drugs were noted, whereas intact animals did react. Of such drugs, psilocin was of particular interest because it produces hallucinations in humans without panic or perceptual disorganization which characterizes the effects of lysergic acid. The tryptomine derivatives were also utilized in this study.

Dr. Herbert Lansdell, in his studies of patients with temporal lobe disease, has arrived at the conclusion that the temporal lobes on the basis of lobectomized patients, do not seem to be especially involved in the types of tasks which are included traditionally in the standard intelligence tests. The temporal lobes seemed to be more involved in maintaining more subtle functions, as detected by verbal and perceptual tests, particularly, in what might be termed the "judgment of the well-adjusted person". After lobectomy, he notes a slight decline in the patient's abilities in this regard.

Dr. John Van Buren is continuing his study on the psychological anatomical correlations in man as well as an evaluation of the effects of new methods for treatment of tumors of the central nervous system. In his study, depth electrodes were implanted in patients subject to seizures of the temporal lobe. Such electrodes were placed in the mesial surface of the temporal lobe and controlled pneumographically. Such electrodes were left in place one to two weeks and the effects of depth stimulation were observed clinically, photographically, and with a polygraph apparatus for determining the autonomic responses. This latter apparatus records blood pressure, skin temperature, electrocardiograms, skin resistance, plethysmograms, esophageal and gastric motility and finally respiration. Dr. Van Buren's studies seem to indicate that clinical automatism was found particularly in or near the amygdaloid nucleus and the hippocampus. Autonomic effects noted by Dr. Van Buren included hypertension, tachycardia, fall of skin resistance, esophageal peristalsis and inhibition of respiration or expiratory apnea.

Emotional responses were few and fear was noted in but two apprehensive patients but not in those previously at ease. In one patient, laughter was elicited with stimulation and was reproduced as part of the automatism.

Autonomic changes, aside from those accompanying automatism, were relatively few and were usually accompanied by a subjective sensation within the viscera and occasionally, a poorly localized sensation of somatic nature. The epigastric sensation was the most frequent.

Along with Dr. Ajmone Marsan, Dr. Van Buren studied a series of 21 ictal episodes associated with metrazol injection in patients with seizures presenting with characteristic visceral phenomena. Here the same stereotype response of the autonomic nervous system was elicited. Variations of the pattern lay predominantly in the failure of a given change to appear or in the sequence of the changes. Most of the aurae were reported by the patients when no significant electrographic activation had occurred and many autonomic changes appeared without electroencephalographic correlates. No autonomic or clinical change was seen to accompany the onset of focal ictal activation either on the surface or in the depth of the temporal lobe, although such changes may appear later as the activity increased in extent and amplitude.

Stimulation of frontal regions might or might not be accompanied by autonomic changes. Thus, most of the changes of the autonomic system appeared against a background of gradually increasing generalized activation with or without a preponderance in the temporal region.

In eleven patients, the orbito-temporal cortex was explored while such patients were undergoing pituitary surgery for metastatic cancer. Constant responses were obtained from the anterior uncus at its junction with the hippocampal gyrus. Here blood pressure would fall. Coincident with this would be a bradycardia. Respiration was inhibited or arrested. This could occur at any stage in the respiratory cycle. Esophageal contractions which appeared could well be independent of the laryngeal component of swallowing. The similarity of this complex to that of stimulation of the superior laryngeal nerves and vagi in lower animals was noted by this observer.

Dr. Van Buren is also continuing his studies on the functional anatomy and pathology of the human visual system in which he now has eleven human cases with normal visual fields and normal retina; one normal monkey and one normal chimpanzee. He has eight human cases and one monkey with lesions of the optic radiation; five monkeys with lesions of the optic tract; seven patients with lesions of the chiasma; two monkeys with the unilateral section of the optic nerve; and one patient with

amblyopia exanopsia.

Dr. Van Buren has now started reconstruction studies of the topographical anatomy of the retinal ganglion cell layer. He has found that in the normal retina such an arrangement of cells is not circular around the macula but forms an elongated oval with its long axis horizontal. The thinner the ganglion cell layer of a given retina, the greater is the ratio of the long axis to the short axis of the oval. He notes that although the optic nerve head appears circular, the actual opening in the ganglion cell layer is an oval with its long axis vertical. In addition, he notes that those ganglion cells over 15 micra tend to have their highest population at the intermediate margin of the more densely populated portions of the ganglion cell layer, i.e., over one cell thick. In the case of a craniopharyngioma, a maximum ganglion cell thickness in the parafoveal area of four cells permitted a visual acuity of 20/20 in one eye; in the other eye with a maximum parafoveal cell layer of three cells thick, the visual acuity was only 20/100. Since the distance between the blind spot and the macula was known both in the degrees of the visual angle and in millimeters of retinal surface, the visual fields and the anatomical plots of the ganglion cell anatomy can be superimposed on the same chart, and Dr. Van Buren noted that while isopters of 5/2000 or larger could be noted by the patient outside the portion of the ganglion cell layer more than one cell thick. This patient had a striking bitemporal visual field defect and there was an outstanding degeneration of the retina in both blind fields. In a patient with an occipital metastatic lesion, there was a demonstrable degeneration of the larger ganglion cells in the blind quadrant and a depression of the margins of the ganglion cell plots in this region. In other words, the margins were closer to the fovea in the blind area than elsewhere. In an initial survey of a monkey retina in an animal with an occipital lobectomy four years before killing again, this transneuronal degeneration of the retina of retrograde character was distinct as demonstrated by the retention of the ganglion cell layer of only two cell thickness as opposed to the normal of seven to eight ganglion cells on the intact side. Thus, it seems clear that Dr. Van Buren has demonstrated that transneuronal degeneration in the visual system not previously reported.

Dr. Choh-luh Li is continuing his study as to the function of the cerebral cortex by studying unitary systems using microelectrodes. Interaction of unitary systems by way of collosal response was utilized by this investigator in a study similar to that reported by Dr. Ajmone Marsan. For example, if a cell became depolarized and excited following a trans-collosal volley, a cell contiguous to such excited cell might become hyperpolarized in response to the same stimulation. This phenomena was noted in the motor area and the somatosensory area and the visual cortex. Dr. Li concluded that this could serve as direct evidence

for the existence of an inhibitory interneuron in the cerebral cortex and further suggested that the inhibition of responses of certain cortical neurons to an electrical stimulus is due to either a depression of the facilitory interneurons or an activation of inhibitory interneurons. Likewise, he postulates that the inhibition of certain cortical neurons to a given pharmacological agent may be determined by such an agent's capacity for either depressing the facilitatory interneuron or activating the inhibitory interneuron.

Dr. Li has also done a study of unitary potentials measured by evoked responses from the striate cortex of cats, following a single electrical stimulus applied to the lateral geniculate body. This response was simultaneously registered by intracellular electrodes and by surface recording electrodes. He noted that the outstanding feature of such experiments was the hyperpolarization following the initial depolarization and excitation of the cortical cell membrane. On the basis of his previous observations, he suggests that the stimulation activated excitatory fibers as well as inhibitory fibers, projecting from the lateral geniculate to the visual cortex. He feels that the inhibitory mechanism in visual function may be more important in somatosensory function since hyperpolarization was relatively rare in the latter. He also notes unitary responses could be coincident with the time of small deflections in the surface-positive waves and that this would seem to rule out the argument that small deflections are due to the arrival of impulses along geniculo-striate fibers.

Dr. Li then turned his attention to the rhythmical oscillations in electrical potentials which can be recorded from the cerebral cortex. He tests the theory of general thought that this activity is generated from dendrites. He found intracellular dendritic potentials difficult to record and this would be anticipated from the studies of Frank, et. al., in which it was shown that the positive spike of the dendritic potential is of such a small area that only the large negative spikes associated with motor neuron could be found. Dr. Li found that prolonged repolarization following the discharge of a surface-negative wave which has so far been thought to be due to activities of dendrites in the superfical layers of the cortex was always accompanied by depolarization and excitation of cortical neurons. Prolonged repolarization following a discharge of a surface-negative wave has been known as the"superficial response of Adrian." Surface positive waves or "deep response of Adrian" which has so far been believed to be due to the activity of cortical neurons and deep dendrites was found to occur in the absence of neuronal activity recorded from intracellular microelectrodes.

Dr. Li now turned his attention to intracellular recording at a cortical level accompanied again by a surface recording electrode. He found the basic mechanism of single cell discharge in the cerebral cortex to be very similar to that in the single muscle fiber. Thus, there appeared to be an intrinsic mechanism and an extrinsic mechanism. The intrinsic mechanism was manifested by rhythmic oscillations of the cell membrane and the extrinsic mechanism by random small potentials. In the epileptic cortical neuron, there was a decrease in the resting potential and an increase in the rhythmical oscillating potential. To understand this further, Dr. Li and Dr. Klatzo are undertaking physiological intracellular recordings in tissue culture material. The technical difficulties are such at this time that they must be resolved before further studies are done.

STUDIES OF INVOLUNTARY MOVEMENTS

With the completion of his basic studies on the stereotaxic device designed by himself, Dr. Van Buren has now entered into an intensive study of the altered physiology and therapy of involuntary movements. In doing such, he is joined by Dr. Ajmone Marsan, Dr. Hertz, Dr. Lansdell, and Dr. Cornman. Studies undertaken on such patients are: the recording of reported sensory results of stimulation; oscillographic recording of evoked activity at the cortical surfaces from stimulation of the basal ganglia; oscillographic recording of areas of spread electrical impulses from stimulation of the basal ganglia; recording of autonomic effects of such stimulation; EEG recording of activity in depth after waking, sleep, and drug-induced sleep; EEG recording of the effect of lesions in the depth upon electrical activity from the scalp. Concomitant with this will be an evaluation of the endocrinological effects of lesions within the basal ganglia and analysis of the psychological effects of such lesions as well as the practical physiological effects of therapeutic ablations as of such ganglia. To date, eight patients have been subject to depth electrode placements in the basal ganglia. Such patients are diagnosed as choreoathetotics, dystonics, Parkinson's syndromes, etc. Multiple port depth electrodes were placed in the thalamus or pallidum in such patients, as well as extradural electrode over the frontal cortex. These investigators now have studied 98 points in the anterior inferior thalamus, the genu of the internal capsule, both segments of the pallidum, putamen, and caudate nucleus. It is of interest that sensory responses have been obtained in nearly all patients. Practically all of these were referred contralaterally to the side stimulated. If the placement is near the optic tract, visual sensations contralaterally or straight ahead may be evoked. But more rarely, (i.e., 2 patients) formed objects have been seen. Somatic sensations have consisted of tingling, tickling, or warmth referred to various portions of the contralateral body. It would appear that brief stimuli within the medial segment of the pallidum evoke a

wide distribution in the thalamus, while conduction of stimuli originating in the inferior portion of the ventralis lateralis of the thalamus is found only to the mesial section of the globus pallidus.

STIMULATION AND ABLATION OF CENTRAL NERVOUS SYSTEM BY RADIO-FREQUENCY ENERGY

Dr. Baldwin, Col. Bach, and their associates have continued their studies on the effects of radio-frequency energy waves on the primate brain. This apparatus has now been adapted so as to study the effects on the chimpanzee as well as the monkey, and a new system has been devised in combination with the Fort Knox Microwave Laboratory so that the effects of radio-frequency on serum protein targets can also be observed. Dr. Baldwin now feels that there is a relationship between the frequency of the output and the clinical or chemical effect. Thus, the chimpanzee responds to a frequency of 240; whereas in the monkey, the frequency range is 388. Electrophoretic changes have been produced in serum albumin in the range of 30 mc. and harmonics of this frequency. He and Col. Bach feel that the dissociation constant of the solvent media play an important part in the protein changes during radiation. Thus, protein in distilled water is not effected while protein in buffered saline was effected. The effect of radiation on the whole animal was inadvertently demonstrated with the first application of the new kilowatt transmitter to the chimpanzee in that this animal died in less than a second. The changes in surviving animals have been carefully studied by Dr. Klatzo and blood-brain-barrier changes continue to occur around the aqueduct in the upper part of the fourth ventricle. Pathological changes are characterized by tigrolysis localized in the pons and medulla. These investigators found the previous aspiration of CSF protects the animal from the more severe clinical effects. The reasons for this at the present time are unknown.

EFFECTS OF DEEP HYPOTHERMIA

Now that the majority of severe intracranial operative procedures are carried out underhypothermia, this Branch has continued its long-term investigation as to the effects of hypothermia in both dogs and higher primates. The study was carried out by Doctors Baldwin, Galindo, and Adamkiewicz. Doctors Galindo and Bucknam have now applied the technique of deep hypothermia to more than 100 dogs. Working in conjunction with Dr. Baldwin, they have shown that esophageal temperatures in the range of 4 to 7° C. can be obtained and maintained without cessation of extracorporeal circulation. They have applied similar techniques to higher primates and it is now clear, at least in the chimpanzee, this will provide a relatively safe background for intracranial procedures. One frontal lobotomy was carried out at a 4° C. in a chimpanzee whose circulation had been arrested

for 45 minutes. The esophageal temperature at the time of this operation was 7°. At this point circulatory arrest was begun and continued in the temperature range of 4 - 7°C. and the animal recovered. These investigators found that blocking the sympathetic system would reduce or entirely prevent the chance of cardiac fibrillation. Selective cooling of the heart may also be done so as to decrease the time required for cardiac arrest. During such a procedure, the brain as might be expected is markedly changed and it appears white and flattened. The vessels usually of moderate to minimal size are now invisible on the surface and the larger vessels appear as fine lines. Dissection at this time is relatively easy and the brain separates along the anatomical lines with great ease. The utilization of such low hypothermia in human procedures is the ultimate aim of this investigation.

VASCULAR PERMEABILITY IN CEREBRAL EDEMA

Dr. Klatzo and his section have continued studies on the vascular permeability of the brain in a variety of situations utilizing intravenous fluorescein labeled serum proteins. He and Dr. Webb Haymaker of the Armed Forces Institute of Pathology have now studied the brains of 63 rats who were exposed to alpha particles at a surface dose of 6,000 rad from a 60 inch cyclotron of Berkeley, California. The cyclotron aperature through which the alpha particles passed was 14 mm. and was of sufficient size to allow radiation of most of the dorsal surface of the cerebellum and cerebrum bilaterally. Following such radiation, the rats were sacrificed at various time intervals. Prior to the sacrifice, the majority of animals were injected with fluorescein labeled serum proteins (FLSP). The major findings of these investigators on such radiated animals was that the primary noxious effect of radiation appears within 24 hours, at a depth band corresponding in intensity to the Bragg curve (see Ophthalmological studies on the lens in radiation). The primary damage within the Bragg zone is exemplified by pyknotic nuclear changes and the appearance of PAS droplets. The first vascular permeability changes appeared at 48 hours after radiation and were demonstrated by the microscopic leakage of FLSP from the capillaries, as well as by a perivascular reaction of the astrocytes in the zone of radiation damage. Extravascular leakage of proteins could be demonstrated microscopically as late as 36 days after radiation, whereas gross injury to the blood brain barrier, as tested with sodium fluorescein, is not detectable after the 18th day. Similar studies were carried out by Dr. Klatzo in primates utilized in the radiofrequency energy study. In animals sacrificed within a few minutes after exposure to the lobe excision "low position", changes in the permeability of the blood brain barrier may be found in the pons, medulla, and extending to the white matter of the cerebellar hemispheres. Occasionally, such changes are found lower and involve the dorsal areas of the cervical spinal cord. Histological changes in the acute experiments were confined

to striking changes in the large tegmental neurons of the pons, medulla, and dentate nucleus of the cerebellum.

In chronic experiments utilizing the radio-frequency energy, Dr. Klatzo found that only a few animals showed changes in the blood brain barrier and these changes were localized in the dorsal portion of the cervical cord. They consisted in severe tissue destruction, mainly in the posterior columns. In such areas, small cystic cavities could be seen. A few such cavities were found in the medulla and pons. The neurons in the vicinity were well preserved.

Dr. Klatzo is continuing, also, his study on pinocytosis (drinking in by cells) in nervous tissue. His observations in vitro , utilizing tissue cultures of neural cells, demonstrated that such an uptake of FLSP by pinocytosis was correlated with the characteristics of the individual cell type, thus it was more active in the microglial macrophages. An uptake of FLSP was also present in the astrocytes, oligodendrocytes, and Schwann cells. Pinocytosis was not observed in nerve cells on the other hand, but was vigorous in the perineuronal satellites. In vivo, the uptake of FLSP by glial cells in an area of edema and in the subarachnoid space showed a striking resemblance to that observed in the tissue culture suggesting that the same mechanism is involved. These investigators feel that their observations emphasized the importance of glial cells as metabolic intermediary structures.

Utilizing the same techniques of fluorescein labelled serum protein (FLSP), these investigators studied the blood brain barrier and vascular responses to allergic encephalomyelitis. They found in animals sacrificed after the occurrence of symptoms, there could be seen fluorescent perivascular exudates without inflammatory cells in the Virchow-Robin spaces. They also found other areas in which there was an intense inflammatory cell response without any evidence of increased permeability of FLSP. Occasionally, such exudates could also be observed within the stroma of the choroid plexus. In some areas, there was a wide parenchymatous FLSP spreading similar to that seen in the edema reported above. Such areas again were observed in the white matter and occasionally were devoid of any inflammatory cells. Animals were sacrificed later in the disease at the time of demyelination and intense astrocytic proliferation and no increased permeability to FLSP was found. In one severely affected animal, FLSP was found in the subpial astrocytes. In a further study of cerebral edema, Dr. Klatzo and Dr. Miquel injected the FLSP intravenously after precipitating localized edema in the white matter by a cooled metal plate reported in the previous Annual Report. The FLSP was also injected into the ventricles and into the subarachnoid space. Again they found a wide distribution of the FLSP throughout the white matter in the first

hours after cold injury. Distribution of this was much larger than the area of the injury and again limited to the white matter. Fluorescence tended to disappear as time progressed; and FLSP was then found within the neuroglial cells. Such inclusions within the cells were strikingly similar to the pinocytotic inclusions observed in the tissue culture cells. After 48 hours, two distinct routes of transport of the FLSP could be recognized; one towards the pia and the other towards the ependyma. The microglial cells appeared to be the main transporters of the material. Following intraventricular or subarachnoid injection of FLSP, no intraparenchymal invasion was observed in animals provided no signs of increased intracranial pressure were present. In animals which tolerated this procedure poorly and developed increased intracrainal pressure, a diffuse green fluorescence was again observed in the superficial band of brain tissue facing the pial and ependymal surfaces.

PERINATAL ABNORMALITIES

Dr. Anatole S. Dekaban is continuing his study in both animal and human material of abnormalities occurring on or about the perinatal period. He has concluded his Atlas of the normal mouse brain at 11 age horizons. He has gone on to produce malformations by x-radiation of such mice. Ninety-eight litters were obtained from irradiated mothers. Approximately 10% of these had major abnormalities, about 25% minor abnormalities, and the rest were free of detectable pathology.

In his study of pregnant women and their offspring at the Walter Reed Army Hospital and the National Naval Medical Center, 4,156 pregnancies and the products thereof were studied in great detail. From such studies of these pregnancies, he found 28 severe abnormalities. Thirteen of these were from complicated pregnancies and five were found in the controls. Of the moderate abnormalities, fifty were found in complicated pregnancies but fifteen in the controls. In the mild abnormalities, 87 of 96 were found in complicated pregnancies.

Congenital malformations, retardation of development, and epileptic seizures constituted the most serious abnormalities encountered. Malformations were the cause of 18 fetal deaths, neonatal or infantile deaths, and a cause of a serious handicap in the 26 surviving offspring.

Dr. Dekaban studied intensively fifteen brains from infants who died in the acute phase following cerebral birth injury. The hemorrhagic lesions he found were grouped by him into six categories: (1) The hemorrhage of massive nature associated with the tear of the tentorium or falx; (2) Massive, focal subarachnoid hemorrhages associated with hemorrhagic

lesions in the brain; (3) Intraventricular hemorrhages; (4) Hemorrhagic infarctions; (5) Direct, traumatic bruising of a part of the brain; and (6) Minor, extravasations of blood in the subarachnoid space. In a study of the infant's occiput at birth and a surviving eight-year old child, Dr. Dekaban drew the plausible hypothesis that rapid extraction of the fetus or prolonged difficult birth in certain positions causes great elongation of the occiput. This, in turn, may result in consequent stretching and compression of the posterior cerebral arteries causing infarction of the occipital lobes. He feels that this may give some insight into the pathogenesis of symmetrical occipital ulegyria.

This Section studied the outcome of 235 pregnancies in diabetic and prediabetic women and matched these with 249 controlled pregnancies. In the diabetic sample, there were 29.9% abortions and previable deaths. In the prediabetic group, there were 20.5% such deaths while in the normal control there was but 12.4%. Stillbirths presented a frequency of 11.5% for diabetic mothers, 5.1% for prediabetic mothers and 1.2% in the normal controls. Neontal deaths amounted to 8.3% of all pregnancies in diabetic women, 1.3% in prediabetics and 3.6% in the normal controls. There were six abnormal surviving offspring in a sample of 157 diabetic pregnancies which amounted to a total of 3.8%. In a normal control sample of 249 pregnancies, there was only one abnormal surviving offspring. The abnormalities detected in such surviving offspring of diabetic mothers included mental deficiency, congenital malformations, birth injury, and seizures. Of those offspring with congenital malformations, two presented with mental dificiency and one with epilepsy. Of those infants born dead, eight had a pulmonary hyaline membrane disease, three had congenital malformations, two had cerebral birth injury, and no gross lesions were found in the remaining three.

In Dr. Dekaban's investigation of etiology, pathology and clinical manifestations of "cerebral palsy", he came to the conclusion that epilepsy of idiopathic type can occur in infants under 1 year of age and frequent daily attacks of "petit mal" occuring under 1 year of age can cause significant and irreversible mental retardation.

Finally, this Section is initiating a study of chromosome abnormalities in patients with congenital malformations, and will be joined in this study by Dr. Michael Bender of the Oak Ridge National Laboratories.

LABORATORY OF NEUROCHEMISTRY

The Laboratory of Neurochemistry's combined program has now been split. The reporting on the Section of Lipid Chemistry under Dr. Roscoe O. Brady is now included in this Annual Report. Dr. Brady has left the section for a year of further training in California, and Dr. Bernard Agranoff is now stationed in Michigan for the coming year. The laboratory will within the next six months be incorporated into the other sections of Neurochemistry of the Neurological Institute and a new laboratory will be formed. In this reporting period, Dr. Eberhard Trams has submitted an acting chief summary of the functions of the Section of Lipid Chemistry. The section reports on nine projects during this reporting period, five of which are directly concerned with biosynthesis of lipids of importance in the central nervous system and of the myelin sheath. The Neurological Institute has maintained in the past and will continue to maintain and expand this activity, in that brain structure is so dependent upon an understanding of lipids and their normal and abnormal metabolism that such studies are unique almost to this Institute. The biosynthetic studies reported by these investigators concern sphingolipids, cholesterol, fatty acids, brain gangliosides, and certain aromatic compounds, particularly the formation of methyl-salicylate. The elucidation of the synthesis of gluco-and galacto-cerebrosides (sphingosine lipids) is of importance in the understanding of the lipid storage diseases which may affect the central nervous system, particularly Gaucher's disease and Niemann-Pick disease. The formation of the glycosidic linkage, the formation of the N-acyl linkage of sphingosine, and the various possibilites for the formation of long-chain fatty acids in the ceramide portion of cerebrosides are studied in this laboratory with the conventional analytical techniques. Such a study utilized the spleens of patients with Gaucher's disease or Niemann-Pick's disease, and in situ formation of the cerebrosides in the spleen was demonstrated. It was concluded, however, by these investigators that the rate of cerebroside formation in Gaucher's disease was not sufficiently elevated to warrant the conclusion that the biochemical lesion in this particular type of lipid storage disease was due to an increased rate in lipid synthesis. These investigators now propose, therefore, to investigate the catabolism of such lipids in patients effected by the disease.

The large amount of cholesterol in the brain and its low metabolic turnover coupled with the fact that it is largely unesterified might indicate a structural function for brain cholesterol and an important role for a synthetic pathway. Dr. Agranoff continued this study on the biosynthesis of cholesterol in collaboration with Professor Lynen; he utilized C^{14}-labelled

precursors which were incubated with tissue preparations and the reaction products were isolated and identified. Pathways previously elucidated in yeast were confirmed in animal tissues. He found the condensing system to make a 30-carbon chain from six 5-carbon precursors and termed this system "Terpene Synthetase." This name was chosen presumably to emphasize the polymeric nature of the product. Dr. Agranoff feels that a chemical resemblance between isopentenyl pyrophosphate iosmerase enzyme-substrate compound and penicillin was found suggesting to him at least an analogous relationship between terpene formation and the formation of penicillin in micro-organisms.

In his summary, Dr. Trams emphasizes that in vivo synthesis of the N-acyl bond in the ceramide portion of sphingolipids is as yet poorly understood. The fatty acid portion in some sphingolipids is represented by fatty acids which have a chain length of more than 18 carbon atoms and the origin of such long-chain fatty acids is unknown. This section undertook an investigation of the origin and activation of such long chain fatty acids, i.e., C 20, since they have found that this seems to fit well into the already established program on the biosynthesis of fatty acids. In such a study, carbon or tritiated labelled precursors were used as substrates for rat brain and/rat liver enzymes. Gas chromatographic analysis was then performed for the identification of the reaction products. Conventional fractionation procedures were employed to prepare the requisite enzymes. Previous studies from this laboratory have demonstrated that Malonyl coenzyme A was one of the key intermediates in fatty acid biosynthesis and this has now been confirmed by several other investigators and the Associate Director would like to indicate a recent review of this in the British Medical Bulletin of the current year on insulin metabolism. Dr. Brady and Dr. Trams have described a method for the chemical synthesis of carbon labelled Malonyl CoA. This and other labelled precursors were utilized in the further study of the reactions necessary for the formation of palmitic or stearic acids. Their observations indicated the enzymes involved were of the sulfhydryl type and that the juxtaposed R-SH groups within the enzyme were an integral part of its active site. In an extremely nice demonstration, these investigators showed that the synthesis of palmitic acid is accomplished by the condensation of one mole of acetyl CoA with seven moles of Malonyl CoA. The search for intermediary products that might be produced during elongation of the carbon chain was non-rewarding, so these investigators concluded that the long-chain fatty acids were formed directly on the enzyme surface without liberation of intermediary products. Further steps to accomplish such were done with the use of tritium labelled triphosphopyridine nucleotide. The observation of the incorporation of tritium into the beta position only, to form palmitic acid was considered by them to be a significant finding, and thought to support the mechanism of fatty acids synthesis postulated previously in this laboratory. These investigators now hope to extend these studies to fatty acids containing

up to 24 carbon atoms. Their preliminary studies have demonstrated that such acids are not necessarily synthesized by the same mechanism.

Dr. Roscoe Brady has undertaken further investigations on the enzymatic carboxylation of acetyl CoA. The objective is learning the mechanism of the biosynthetic pathway for the formation of malonyl CoA and related compounds. As pointed out before, malonyl CoA was the key intermediate in fatty acid synthesis. These enzymes were isolated from liver and heart which catalyzed the carboxylations in the presence of ATP and bicarbonate, and were shown to be biotin dependent. Dr. Brady further showed that the pyruvate or phosphoenol pyruvate would stimulate the carboxylation reaction and that manganese was required. Carboxylation of propionyl CoA proceeded at a much faster rate than the reaction with acetyl CoA and the requirements for the two reactions differed in some respects. However, no clean separation of the propionyl from the acetyl CoA carboxylase was achieved. Further investigations will be undertaken to attempt to separate these two carboxylases.

In the disorders of the nervous system known as the amaurotic idiocies, it has been found that gangliosides accumulate in the central nervous system of children so effected. The study on the structure and biosynthesis of brain gangliosides was hence undertaken in this laboratory. A new method was developed for the isolation and purification of the gangliosides from brain. Highly purified preparations were analyzed by standard procedures for their component building blocks. In agreement with Klenk these investigators established that the ganglioside was composed of sphingosine, fatty acids, glucose, galactose, N-acetyl galactosamine and N-acetyl neuraminic acid, however, no simple repeating unit could be isolated. Sedimentation studies indicated that the polymer was not bound through co-valent linkages; that it was indeed an aggregate in aqueous solution. Enzymatic degradation revealed substances in many ways similar to the so-called cytosides. Isotopically labelled precursors in the ganglioside showed that the total synthesis and turnover of such material was very slow.

Dr. Eberhard G. Trams, Dr. Robert A. Resnik, and Dr. Richard L. Irwin, the latter two from the Branch of Ophthalmology and the Branch of Medical Neurology have collaborated in a study to see if such gangliosides could be used in an attempt to identify certain macromolecular glycolipids as cell receptors in neural tissue. In such a study, gangliosides were subject to simultaneous chemical and physical analysis in order to identify the shape, size, and charge of the molecules. The interaction of these materials with quaternary amines and other central nervous system active drugs was studied by bio-assay on isolated organs and in whole animals. The initial studies of these investigators indicate that the gangliosides seem to be able to antagonize the

effects of certain of the quaternary ammonium compounds. An investigation regarding the possible role of these and related materials as cell receptor sites has hence been initiated. The possibility that glycolipids are able to sequester a number of quaternary ammonium compounds such as curare and thus apparently exert a blocking action of some of these compounds in biological systems has important implications. Attention to this has recently been directed in the public press through the work of Nachmannson of Columbia University. Doctors Roscoe O. Brady, W. Siegelman, and M. D. Lane continued their investigations on the mechanism of formation of aromatic compounds, the objective being to discover the enzymatic pathway of the formation of methyl-salicylate which arises apparently through the condensation of 2-carbon fragments derived from acetyl-CoA. Standard enzymological methods as well as tracer techniques were used. It appears that labelled acetate is a preferential precursor for the biological formation of compounds containing benzene rings. In plants at least, a biological route exists for the benzene ring formation other than that by way of sedoheptulose, quinic, and shikimic acids. These investigators feel that they have established a preferential conversion of labelled acetate to 6-methyl salicylic acid in vitro. Insight into such mechanisms could give an indication as to the pathway of formation of certain quinones such as vitamin K and coenzyme Q. This project will be discontinued until Dr. Roscoe O. Brady's return from California. In the meantime Dr. Eberhard G. Trams has continued his study on the effects of hydrocarbons on enzyme synthesis. His objective is to ascertain the mode of action of a number of carcinogenic hydrocarbons which have been shown to induce the synthesis of certain microsomal enzyme systems also known to inhibit other such enzyme systems. It was found that the activity of certain microsomal enzymes seemed to decrease in the course of chronic administration of these hydrocarbons. Other enzymes seem to respond with increased activity in what appeared to be compensatory mechanism. The principal declining system was the N-demethylating system and the hydroxylating activity of the liver microsomes. On the other hand, glucuronyl transferase and glutathione reductase and UDPG-dehydrogenase systems all appeared chronically stimulated. Dr. C. S. Spyropoulos of the Laboratory of Neurophysiology and Dr. Roscoe O. Brady reported in 1959 that oxidating agents were capable of causing a decrease of the resting potential of a nerve fiber with a concomitant spontaneous continuing firing. This suggested to these investigators that the membrane of living nerve tissue normally contains a component in the reduced state. In efforts to determine whether or not redox reactions participated in excitation and impulse conduction, the effects of conenzyme Q and derivatives thereof were investigated on chronically stimulated unmyelinated nerve fibers. The project is in the preliminary stage and will be continued upon Dr. Brady's return from California. Finally, Dr. B. W. Agranoff with Dr. Harry Eagle of the National Institute of Allergy and Infectious Diseases undertook an investigation of the function of inositol

as a vitamin. Inositol, labelled with radioactive tracers, was incubated in the presence of cells grown in culture and the radioactive products analyzed for inositol content. The concentration of inositol was determined by microbiological and chemical methods and these investigators found inositol-dependent mammalian cell types appear to require inositol for the production of structural phosphatidyl inositol. Inositol was also found in a bound form in protein and nucleic acid fractions. In addition, however, certain cell types were able to synthesize inositol to support growth in deficient cell types when the two culture vessels were separated by a dialysis membrane. This present study indicated that particular somatic cell types may possibly furnish constituents for other cells.

LABORATORY OF NEUROPHYSIOLOGY

With the reorganization of the Neurological Institute, it was the decision of the program directors that the Laboratory of Neurophysiology should allow area sections to continue to act as a single entity under the present Laboratory Chief, Dr. Wade Marshall. However, those areas in the province of each of the two Institutes were clearly demarcated with regard to space, budget, and personnel. The sections of Spinal Cord Physiology, and Special Senses, thus fall in the scope of this annual report. The Laboratory Chief, Dr. Marshall, will make his report concerning the integrated laboratory to both directors of each of the two Institutes. Upon completion of the new facilities, the two sections, Spinal Cord Physiology and Special Senses Physiology, will form the nucleus of a new Laboratory of Neurophysiology within the National Institute of Neurological Diseases and Blindness.

Three broad areas of research endeavor are represented in these two sections. First, the major efforts of both sections has been used to further understand the basic mechanisms underlying the generation of impulses and the mechanism of nerve excitation using diversified techniques dependent upon the section's approach to the problem. Second, using the auditory pathways, investigations were undertaken to understand integrative mechanisms of cellular sub-stations utilizing bilateral stimulation combined with microelectrode recordings within such sub-stations. The third study has just been initiated and is directed towards possible chronic changes that might occur in single cells of the central nervous system associated with altered sensory input, i.e., cellular changes possibly associated with learning.

GENERATION OF IMPULSES AND MECHANISMS OF NERVE EXCITATION

Dr. Ichiji Tasaki and Dr. C. S. Spyropoulos have continued their studies concerning the basic mechanisms of nerve excitation, including impulse generation, conduction and their consequences. The highly productive work of this section over the past years has now been incorporated into a review which includes all the concepts on excitation arrived at by these investigators. This has been submitted and is to be published in The Physiological Reviews. During the current year, these investigators have turned largely to the radioactive isotopes to further elucidate the mechanism of nerve excitation, and in this study have used multiple intracellular wire electrodes, intracellular injections of isotopes, and perfusion of the intracellular compartment. In such studies, the squid giant axon was used. By means of gamma-spectrometry, simultaneous measurements of radioactive sodium and potassium were made and combined with electrophysiological techniques to study ionic movements in the squid giant axon. Similar radioactive tracer techniques were used with artificial membranes of the cation exchange type. A highly ingenious rotating fractionator was employed for measuring tracer movements during prolonged action potentials. The principles governing the movement of tracers across animate and inaminate membranes, and the relation of such tracers to the stable species were interpreted on the basis of thermodynamics of irreversible processes. These investigators feel there is a justification of such proposed principles as applied to nerve membranes in that similar results were obtained by study of movement of tracers across the cation exchange resin membranes. Radioactive tracers employed were K^{42}, Na^{22}, Ca^{45}, P^{32}, Cl^{36}, S^{35}, tritiated water, Cs^{134} and the various C^{14} organic molecules. In the resting state, the giant squid axon lost intracellular tritiated water with a time constant of 0.5-1.0 minutes, whereas the corresponding figure for the Ca^{45} divalent ion was approximately twenty minutes. When C^{14}-labelled thiourea and urea were measured there was a time constant of 30-100 minutes, whereas for all univalent cations, the time constant was between 5 and 15 hours. For anions examined by these investigators, i.e., chloride, sulfate, and phosphate, and for large neutral molecules such as sucrose and starch, the time constant was greater than 50 hours. These investigators felt that calcium, choline, and phosphate were chemically bound with the axoplasm and possibly also, in part, with the membrane.

If the axon now was repetitively stimulated, the movement of the unbound cationic tracers such as sodium, potassium, cesium, and guanidinium was accelerated; similarly, if calcium and magnesium concentrations in the bathing fluid were reduced or the potassium concentration increased, there was also an increase in the rate of loss of these unbound cationic tracers. None of the

neutral or anionic tracers were found to be affected by either repetitive stimulation or by potassium depolarization. Based on such findings, these investigators postulated that the resting squid axon membrane had a fixed negative charge and that the membrane remained negatively charged during its activity.

Using the rotating fractionator, these investigators collected samples of sea water flowing by such a squid giant axon at different intervals during a prolonged action potential. Their preliminary results indicated that both K^{42} and Na^{24} were released from the axon both at the beginning and at the end of the prolonged action potential. There did not appear to be any profound preference of one of these cations over the other. Such collections, however, were at millseconds intervals.

In previous studies from this Section, Dr. C. S. Spyropoulos has demonstrated that the nickel divalent ion will lengthen the action potential. These investigators applied nickel chloride to the toad single node preparation and found it was possible to initiate in an all-or-none manner, an action repolarization after subsequent application of a small amount of K-rich fluid. The application of a calcium divalent rich fluid to the node during plateau of prolonged action potential was found to abolish such an action potential. Similarly, a sudden temperature rise applied during such a plateau was also found capable of abolishing the action potential. These investigators came to the conclusion, based upon these findings, that competition between univalent and divalent cations at the negative sites on the membrane play an essential role in the two stable states of the nerve membrane. Using the squid axon membrane, these investigators reconfirmed the finding that an injection of tetraethylammonium chloride along a long stretch of the axon will prolong the action potential from its normal value of approximately 1 millisecond to as long as 100 milliseconds or more. However, when only a short portion of the axon was treated with TEA solution, there was only a slight prolongation of the action potential. This was attributed by these investigators to the effect of the electrical current flowing between the normal-untreated and TEA-treated portions of the membrane which brings about a premature termination of such a prolonged action potential. This process of abolition of the action potential was found to propagate along the length of the axon. Hence, these investigators feel that the mechanism of initiation of repetitive firing of impulses at such a boundary was clarified. Similar cationic and anionic studies were carried on with the Nitella cell. The time course of loss of intracellular K^{42} during activity was found to roughly parallel the impedance loss associated with such an action potential. They failed, however, to detect a similar movement of Na^{24} tested by the same technique. These investigators suggest that in this organism, the major portion of intracellular Na^{24} is in a bound form. The influx and efflux of the anion Cl^{36} was investigated in two species of Nitella and it was concluded by these

investigators that this anion was not involved in the primary steps of action potential production. In such studies, these investigators were also able to develop a technique for space clamp of the Nitella internode. Using the Aplysia ganglion cells, these investigators demonstrated two stable states in that ganglion cells were found whose membrane potential stabilized periodically, presumably for intrinsic reasons, at the active state for several seconds. Such a potential could be abolished by inhibitory impulses which impinged upon the cell. These investigators found that they could simulate such inhibitory potentials by passing an inward current into the cell. In such studies, these investigators were aided by Dr. Toshihiko Oikawa from Japan, and Dr. Angelique A.-Chalazonitis and Dr. Nicholas Chalazonitis from France.

Simultaneously, in the Section of Spinal Cord Physiology, studies on the generation of impulses of nerve cells were being undertaken by Doctors Karl Frank, Phillip Nelson and Toshihiko Oikawa, a visiting scientist from Japan, in that Section. Within the medulla oblongata of the goldfish is found a pair of giant nerve cells which have been termed the Mauthner cells. These cells consist of two long dendrites and an axon passing down the contralateral side of the spinal cord to the tail muscle of the opposite side. This is a highly specialized nerve cell which lends itself readily to electrical measurement and could provide a model for other less accessible nerve cells. In the study of such cells, intra- and extracellular action potentials were recorded during anti- and orthodromic stimulation. Membrane excitability was measured by direct excitation through a glass micropipette. The relationship of the extracellular potential fields to the cell structure was studied by using marking techniques and histological studies. Antidromic excitation of one cell by stimulation of its axon near the tail produced inhibition of the other Mauthner cell presumably by way of axon collaterals. Repetitive firing of this single cell resulted in multiphasic potential fields which were mapped by these investigators. Reconstruction of the activity of such a cell from the study of these fields indicated that a large monophasic negative spike was associated with firing in the axon cap; the positive-negative potential and monophasic positive potentials fields were concluded by these authors to be the result of a passive depolarization of the cell soma and dendrites respectively. Thus, it appeared that active propagation ended at the axon hillock and the invasion of the soma and dendrites was electrotonically propagated. These investigators, however, did not see in such extracellular fields, an inflection point in the spike potential as is seen in many other nerve cells including spinal cord cells as reported previously by Doctors Karl Frank and Michelangelo Fuortes. Resting and action potentials were measured intracellularly by these investigators; the resting potential of the cell soma being 70 to 80 millivolts. The action potential, however, was but 30 to 40

millivolts showing, therefore, a reduced response consistent with electrotonic propagation.

Similar maps of the external electrical fields produced in the spinal cord by activity of single motor neurons have been carried out by Doctors Karl Frank and Phillip Nelson. The axon of a single motor neuron is isolated and antidromically stimulated. Extracellular potentials produced by this cell at a distance, however, were smaller than the random electrical activity of other cells as well as the background random noise generated by either the microelectrodes or the amplifiers. Using techniques for augmenting signal to noise ratio, maps have been prepared for both the compound AB-spike and the simple A-spike of the spinal motor neuron. These investigators found that the latencies of the peaks of both the A- and B- components increase with distance from the cell, and that the conduction velocity of both waves is the same within the limits of precision of the experiment. This was at variance with the previous findings of Fatt, who had formerly observed such an increasing latency for the B-spike and had interpreted it as indicating active conduction in the dendrites. However, since the A-spike is probably not conducted in the dendrites and has the same apparent conduction velocity, this interpretation is unjustified. Doctors Frank and Nelson had an alternative explanation: that increasing latency with distance represents decrementing electrotonic spread over the dendritic tree. Thus, they found that peaks of both A and B spikes were nearly synchronous over a region of 5 to 100 microns for the A, and 100 to 200 microns for the B. Although these are both centered about their respective maxima, the two regions are not coextensive, but are displaced by about 200 microns in the dorsal-ventral direction. The B-spike is followed by a positive after potential while the A-spike is not.

Dr. Wilfred Rall in the National Institute of Arthritis and Metabolic Diseases has computed external fields around a single firing nerve cell to be expected in a volume conductor such as the spinal cord using different sets of assumptions. He comes to the conclusion that around such a structure as a motor neuron, predominantly negative signals would be recorded. Correspondingly, positive signals would be confined to very small regions, indeed, around the dendrites. Doctors Frank and Nelson's experimental results verify Dr. Rall's prediction; predominantly negative spikes were recorded. Consideration of these external potential fields suggests that the antidromic spike activity dies out in the soma or bases of the dendrites. Finally, Doctors Rall, Frank, and Nelson conclude that an after positivity, recorded extracellularly, does not necessarily indicate that active conduction has proceeded beyond the nearest active membrane.

SENSORY INTEGRATIVE MECHANISMS

In Dr. Karl Frank's section, Dr. Phillip Nelson and Dr. S. D. Erulkar have undertaken a study of sensory integrative mechanisms utilizing the auditory system of the cat. In such a study, clicks and tone envelopes were delivered to the cats' ears. Microelectrodes were then inserted into the inferior colliculus or medial geniculate body and into individual cells of such structures. Both synaptic and spike potentials were recorded as responses to various patterns of auditory stimuli as well as to currents delivered through the micropipettes. These investigators found that both the inferior colliculus and medial geniculate have cells in which both excitatory and inhibitory post-synaptic potentials could be recorded. Polarization of the cell membrane revealed a direct inhibitory process in both collicular and geniculate cells. When the usual burst type response to external stimulation occurred, an inhibitory component to the synaptic activation could be seen usually. Some units in the colliculus could be seen to respond to a click in one ear with an excitatory post-synaptic potential and a click to the other ear would result in an inhibitory post-synaptic potential. By varying the interval of the stimulating source, the interaction of synaptic potentials from the two ears provides a precise mechanism for comparing the time of arrival of a click. AB-spikes like those of motor horn cells were clearly observed in some of the cells penetrated. Potentials similar to those associated with Renshaw firing in the spinal cord have also been seen occasionally. Other studies will be carried out to see if an intranuclear integrative mechanism analogous to the Renshaw loop of the spinal cord can exist at other levels of neural activity.

INTRACELLULAR ELECTRICAL CHANGES ASSOCIATED WITH LEARNING RESPONSES

Learning has been commonly thought to involve primarily anatomical changes in groups of nerve cells or their porcesses. Accordingly, mapping of cortical areas following conditioned stimuli have occupied much of the time of the Anatomical Laboratory of the University College, London, under Dr. J. Z. Young. Doctors Frank and Nelson suggest that such changes also imply that individual cells could have been physiologically modified even if this only involves changes in their connections. In this study which is projected largely into the future, these investigators plan to apply prolonged series of stimuli to peripheral nerves on one side only of a chronic spinal cat, in an attempt to produce measurable changes in the spinal reflexes of the two sides.

If such reflex changes can be produced, then the preparations will be examined with microelectrodes to determine which cells have been altered and in what way. These investigators plan to initiate such a study using very young cats. In their initial studies, techniques are being explored for use in perinatal animals. These investigators plan to apply repetitive joint flexion as a means of producing a steady unilateral sensory inflow. If such a study demonstrates reflex changes, and subsequently, intracellular changes, these investigators plan to study such material jointly with the Laboratory of Neuroanatomy to further elucidate anatomical substrata of such changes.

LABORATORY OF BIOPHYSICS

The Laboratory of Biophysics is continuing to investigate the ionic movements fundamental to the initiation and subsequent propagation of the nerve impulse.

This, as in the past, is a group of studies developing mostly from the voltage clamp concept and its application to the squid giant axon. These now involve: (1) the rather complex measurements of the ion flows across the membrane without the complications of excitation and propagation; (2) extension of these investigations to a lobster axon; (3) the measurements of radioactive tracer fluxes during the times of the principal ionic current flows across the squid axon membrane; (4) investigations of ionic permeability concepts by mathematical analysis and computations with particular emphasis on non-linear differential equations; (5) the development of similar mathematical models for passive iron electrodes; and (6) measurements of the effect of stereospecificially tailored aminoalcohol derivatives on the electrical activity of the single node in terms of threshold and action current parameters.

In some of these studies, Dr. K. S. Cole notes the collaborations of the Naval Medical Research Institute; the Biophysics Department of Purdue University; the Physiological Laboratory at Cambridge University; Technische Hochschule, Darmstadt; the Computer Laboratory of the Bureau of Standards, and the Marine Biological Laboratory, Woods Hole. These studies resulted in ten publications of fundamental importance in the understanding of ionic transport in the initiation and transmission of the nerve impulse.

Cognizance is taken of the Laboratory Chief's anxieties as to the continued productivity of this Laboratory within but six modules of space. Upon completion of the new facilities, this laboratory will be expanded to sixteen modules. Some studies in physics and physical chemistry and the use of pharmacological and radioactive tracer techniques may then be initiated or extended. The Scientific Director, however, is cognizant that this does not relieve the immediate situation. It is hoped that temporary

adequate facilities may be found within the next year. To secure some flexibility within this Laboratory, initial investigations into these possibilities are already underway.

VOLTAGE CLAMP TECHNIQUES

In a voltage clamp, the ionic current flow is measured after a sudden change of the membrane potential. This potential is measured between the internal micropipette and nearby external reversible electrode and is maintained and controlled as required by an electronic control system. This system computes, produces, and measures the necessary flow of membrane current between other internal and external axon electrodes.

Doctors J. W. Moore, R. E. Taylor, and Cole have reviewed the voltage clamp concept and made critical surveys of the adequacies and inadequacies of its application to the squid axon. Doctors W. K. Chandler and Cole have extended this work by calculations of the stability of the membrane potential and its distribution along an axon. They show the conditions for a small patch to be under uniform control and for a region of a long axon near the potential electrodes to be under adequate control. Mr. L. Binstock and Dr. W. J. Adelman, Jr., have continued development on the original equipment and also produced a simpler more portable version of it. Dr. Moore with Doctors F. Julian and D. E. Goldman, at the Naval Medical Research Institute, are developing methods for measuring individual ion permeabilites of other non-myelinated axons. To do this, these investigators are using electronic feedback and/or sucrose gaps to minimize the external leakage and thereby provide an accurate measurement of the membrane potentials without the usual requirement of an internal electrode. Potential control of the membrane with current fed longitudinally also appears feasible. So far these investigators find that the sucrose insulation gaps used with a lobster giant axon are very effective for obtaining membrane potential and current data.

VOLTAGE CLAMP EXPERIMENTS

Doctors Cole and Moore showed that although the Hodgkin-Huxley empirical expression for the potassium ion current is reasonably satisfactory for the usual range of axon phenomena, it is however quite inadequate to express the delay in the onset of this current after even a moderate hyperpolarization of the axon membrane. Doctors Moore and Adelman have completed studies of the internal sodium ion concentration as measured by the sodium potential of the membrane. Doctors Adelman and Taylor are reporting that the leakage current (which appears in addition to the large, slow, rectification currents of sodium and potassium ions under voltage clamp conditions) also showed some rectification-- but with a time constant of less than 100 microseconds.

This leakage current appears, however, not to be dependent upon an inward movement of sodium, potassium or chloride.

To further understand ionic transport across such membranes, Doctors U. Kishimoto and Adelman used detergents which were either cationic, anionic, or non-ionic. All such detergents increased the leakage current of the membrane, cationic being the most powerful, the anionic second, and finally, the non-ionic. Only the cationic detergents irreversibly reduced both the resting and the action potentials. Differences among effective concentrations of these three types of detergents suggest that the membrane is negatively charged.

Doctors Taylor and Chandler investigated bridge techniques to determine the high frequency alternating current impedance of the squid axon membrane. From this, Dr. Taylor made an attempt to measure the membrane series resistance and the preliminary data are still undergoing analysis.

COMPARISON OF MEMBRANE CURRENT COMPONENTS AND RADIOACTIVE FLUXES

Dr. Adelman, with Dr. L. J. Mullins and Dr. R. A. Sjodin of Purdue University, utilizing the giant axon of the squid under voltage clamp conditions began a comparison of the membrane current components with the outfluxes of radioactive sodium and potassium ions. Their preliminary results indicated that large current components exhibiting delayed rectification (described previously by Hodgkin and Huxley) could be accounted for by the tracers to within 15% for the sodium current, and 30% for the potassium current.

A radioactive sodium loaded axon was pulsed for short durations and low frequencies to the approximate value of the normal membrane potential. Outward early membrane current resulted. Corresponding sodium efflux was determined by collecting the effuent radioactive sodium into sodium- "free" external sea water which was rapidly circulated past the axon. A similar study was done with radioactive K^{42} in which axons were pulsed with longer duration pulses and low frequencies.

These investigators conclude that the more satisfactory and more complete experiments will probably provide independent evidence to support the Hodgkin-Huxley interpretation of voltage clamp data.

MATHEMATICAL ANALYSIS AND COMPUTER INVESTIGATIONS

Doctors R. FitzHugh and Chandler are continuing their analysis of the properties of the Hodgkin-Huxley equations using phase plane concepts and the Berkeley analogue computer, as well as the digital computers of the NIH and the National Bureau of

Standards. This is an attempt to combine the Hodgkin-Huxley equation with the cable theory. In doing so, these investigators have demonstrated that as the linear leakage conductance is increased, the velocity of propagation decreases. When such a leakage conductance is greater than 8.9 mmho/cm.^{2}, a propagated action potential is impossible. The curve relating conduction velocity to leakage shows two velocities for each leakage conductance, one of which is probably unstable.

Dr. FitzHugh at the present time is working in the Electrochemical Department at Darmstadt, Germany with Professor U. F. Franck. They are utilizing mathematical models of passive iron electrodes, and are developing differential equations for electrodes in sulfuric acid and in nitric acid. In sulfuric acid, such equations produce the stable current oscillations obtained experimentally under voltage clamp. Two variables of state were noted in the sulfuric acid iron electrode, the first being the surface area covered by the oxide film, and the second being the pH of the electrode surface which interacts with such an oxidation.

Studying the Ostwald-Lillie model of nerve which is iron in nitric acid, he has found three variables of state: (1) the surface area covered by the oxide film; (2) the electrode potential; and (3) the nitrite concentration on the electrode surface. The nitrous acid enters into an autocatalytic reaction which contributes a current component of its own having a dynatron characteristic.

RELATION OF ACETYLCHOLINESTERASE INHIBITORS AND NERVE ACTIVITY

This is an investigation by Mr. E. R. Whitcomb with Dr. S. L. Friess of the Naval Medical Research Institute of the effects of stereospecifically tailored aminoalcohol derivatives on the electrical activity of the single node in terms of threshold and action current parameters. This is another study of approach to the action at macromolecular levels, of the strength and nature of drug-tissue interaction, similar to the study of Dr. Eberhard Trams and Dr. Richard Irwin using gangliosides and anticholinesterase. In this study a single frog sciatic nerve fiber was isolated and mounted for recording from a single node and the node stimulated directly once per second. Time course and amplitude of the threshold and action current of the node are studied as functions of concentration and molecular structure of the blocking compounds added to the Ringer's bathing medium. In this study, both the cis- and trans tertiary benzilate compounds were used. The cis-member was three times more effective than its trans-counterpart in attenuating the action current and increasing the threshold. However, the trans-produced a greater degree of irreversibility as indicated by the amount of recovery of the action and threshold after replacing the drug with Ringer's solution. In the second study, the drugs Tropine-O-chlorophenylacetate and 4-tropine chlorophenylacetate were used.

Tropine-O was five times more effective than the 4-tropine in attenuating the action current and increasing its threshold however, the tropine-O also produced a greater degree of irreversibility. The third set of compounds used were tropine diphenylacetate and 4-tropine diphenylacetate. Here the 4-tropine diphenylacetate was five times more effective than the tropine diphenylacetate in attenuating the action current and increasing the threshold. Both produced approximately the same degree of irreversibility. Thus, the order of effectiveness of the entire group was: cis-tertiary benzilate>4-tropine diphenylacetate > trans-tertiary benzilate > the tropine O-chlorophenylacetate > tropine diphenylacetate > 4-tropine O-chlorophenylacetate.

LABORATORY OF NEUROANATOMICAL SCIENCES

The Laboratory of Neuroanatomical Sciences has undergone many personnel changes. Dr. William F. Windle is now the Assistant Director of the Institute. Dr. Sanford Palay has been appointed the Laboratory Chief. He will continue as Chief of the Section of Neurocytology. The Section of Perinatal Physiology in Puerto Rico has been shifted from the Laboratory of Neuroanatomical Sciences to the Office of the Assistant Director. Dr. Lloyd Guth has been appointed the Acting Chief of the Section on Development and Regeneration succeeding Dr. Windle in this position. In anticipation of new facilities, the Scientific Director and Dr. Palay will attempt recruitment in the area of experimental embryology within the next six months. Investigations in this Laboratory include: ultrastructure of neural tissues, coordinated approach of investigations in the special senses, in particular, those observed by the 8th cranial nerve, enzymatic studies of cellular fractions, studies on the interrelationships of neurons and glia, the vascular tree of the brain, studies on artifact versus pathology, and studies on regeneration and reinnervation with emphasis on selectivity of pathways and specificity of nerve terminations.

STUDIES IN THE ULTRASTRUCTURE OF THE NERVOUS SYSTEM

At the time of this report, Dr. Palay has completed a study on the first successful electron microscopic examination of secreting neurons, utilizing the nerve cells of the preoptic nucleus of the goldfish. He found such cells to display a marginated Nissl substance, eccentric nucleus, and a central zone containing the Golgi complex, mitochondria, neurosecretory droplets, and multivesicular bodies. The secretory material appears in the form of dense droplets of two sizes, 0.1μ and 1μ or larger. He found no intermediate stages between the two sized droplets. Each droplet was enclosed in a single membrane, 60 A thick. It is suggested that the small droplets arise primarily in the cisternae of the Golgi complex, whereas the large droplets originate by gradual transformation of the multivesicular bodies. The distinction between the two types of droplets may indicate, therefore, a different chemical composition and/or different functions.

A comparative study of ependyma in fishes and rats was initiated by Doctors Milton Brightman and Sanford Palay. They found that the ependymal cells in the rat are nearly filled with fine filaments oriented in a roughly spherical fashion about the nucleus. In the goldfish, however, these filaments were infrequent and instead, the cytoplasm was occupied by large numbers of tubules and vesicles. The ciliary apparatus was also different between the two species. In the rat, the basal corpuscle of the

cilia were fitted with a bundle of short thin filaments that extended at a slight angle from the corpuscle. In the goldfish, each basal corpuscle was fitted with a pair of long, cross-striated bands consisting of fine filaments and extending from the basal corpuscle to a plane perpendicular to the longitudinal axis of the cilium. Apparently, here they insert in the terminal bars of the lateral cell border.

Studies on myelinated neurons in the 8th nerve ganglia were carried out by Dr. Jack Rosenbluth and Dr. Palay. These studies have now been completed and extended to the corresponding cells in the spiral, and vestibular ganglia of the rat. In this mammal, the myelinated neurons had 5-10 lamellae of myelin, whereas in the goldfish, the sheaths could be 50 or more lamellae thick. Studies have been initiated with Dr. Mary Grillo on the autonomic ganglia in the stomach and intestine, and with Dr. Wolfe on the fine structure of the developing dorsal root ganglia. in the chick.

ENZYMATIC STUDIES ON CYTOLOGICAL FRACTIONS

Dr. Robert Albers and Dr. Larry Embree continued their studies as to the role of gamma aminobutyric acid in the metabolism of the central nervous system. In so doing, attention has been directed to the enzyme, succinic semialdehyde dehydrogenase, which was first described by them in 1959. This enzyme has now been purified from the monkey brain and they are studying its properities. They find the affinity of the enzyme for the substrate considerably exceeds the original estimate and they feel this can account for the extremely small concentration of this enzyme present endogenously in the brain. They feel that several properties of this enzyme in relation to the mechanism of action of aldehyde dehydrogenases, as well as the interaction with other brain metabolites which have neurohumoral associations, are of interest.

PATHOLOGY OR ARTIFACT

Dr. Jan Cammermeyer is continuing his studies in an attempt to standardize techniques of pathology so as to minimize artifactual contaminants. In a study on volumetric and histologic variations in the spinal cord, he finds uniform and excellent histological results were obtained by the use of a rapid coagulant fixative such as Heidenhain's Susa solution or p-toluene sulfonic acid, provided the autopsy was delayed for four hours. In his discussion of the so-called "dark" neurons, Dr. Cammermeyer comes to the conclusion that these are mostly artifactual and could be avoided by the techniques developed herein. A series of experiments were performed to either avoid or produce such neuronal staining. A hypothesis was formulated by this investigator that these cells are formed by post-mortem severance of delicate attachments between the membranes and their surroundings. The

arrangements of cells and vessels of the central nervous system from various mammalian species were also studied extensively. A study of the oligodendrocytes as well as other cells of the central nervous system showed again that proper fixation and delayed autopsy was important. It was found that the size of the oligodendrocytes varied with both the region and the species examined. They tended to have an exclusive perivascular arrangement near neurons, and perivascular arborizations of both gray and white matter. From these studies, Dr. Cammermeyer concludes that the older concepts about the functional significance of oligodendrocytes may well be reputed. He presents a new hypothesis that these cells form juxtavascular clusters which may be concerned with the intrinsic control of the blood flow to the neurons. In this respect, it is of interest that Dr. Cammermeyer noted that neurons in all regions contact blood vessels for varying lengths.

REGENERATION AND REINNERVATION

The Specificity of End Organs

Dr. Lloyd Guth and Dr. C. J. Bailey and Dr. Karl Frank (Laboratory of Neurophysiology) as well as investigators from Boston University School of Medicine and Columbia University School of Medicine have in the past 2-3 years carried out a fascinating study in which attempts have been made to reinnervate on the one hand, sympathetic nervous system structure by the other sympathetic nerves which ordinarily do not subserve the same function, and in a more difficult situation of using a parasympathetic nerve to reinnervate what is ordinarily a somatic function. In the first such study, non-pupillary sympathetic nerves are utilized to see if they could mediate a pupillary reflex. In this study, transection of the sympathetic rami T1-T3 is carried out to allow collateral sprouting from the T4-T7 sympathetic segments (which ordinarily do not carry pupillary rami) to see if such sprouts could innervate the pupillary postganglionic cells of the superior cervical sympathetic ganglion. In this study, they found that pupillary tonus was restored by the foreign nerve fibers, but that these fibers did not participate in sympathetic pupillary darkness reflexes. In the other study, anastomosis of the proximal recurrent larygneal nerve and the distal phrenic nerve segment was attempted; and they demonstrated that the recurrent laryngeal nerve could indeed restore function to the denervated hemidiaphragm of the rat. In the monkey, the recurrent laryngeal nerve transmits functionally effective inspiratory action potentials to the denervated hemidiaphragm only during asphyxiation. This has significance in human diseases which are characterized by destruction of phrenic motor neurons. If reinnervation of the diaphragm by the recurrent larygneal nerve could be established, such patients might survive without the assistance of an artificial respirator. The experimental phase of this

program with animals has now been completed and the initial clinical aspects are being studied in Boston.

Studies on the regeneration of peripheral nerves have been planned by Doctors Lloyd Guth and Jerald Bernstein to see if such peripheral nerves regenerate selectively to appropriate endings or randomly toward any termination. This is a projected experiment in which the sciatic nerve of the rat will be transected and resutured, and after regeneration is complete, isometric tensions developed in the soleus and plantaris muscles upon stimulation of spinal roots L4 and L5, will be studied. A difference in the proportions of the L4 and L5 fibers innervating such muscles would be evidence that the regeneration was not random. The second approach will be that of the cervical sympathetic trunk of the cat which again will be transected and sutured. After regeneration is complete, the rami of T1 to T7 will be stimulated. If T1 to T3 dilate the pupil, and T4 to T7 constrict the ear vessels, this would be evidence that the regeneration again had not been random. Four other such experiments are anticipated by these investigators, all directed toward the determination of a specificity between site of origin and the end receptor.

Dr. Earl Feringa has also undertaken a study in the regeneration of the peripheral nerve to evaluate nerve grafting procedures as a method of obtaining regeneration across relatively long gaps in peripheral nerves. In this study, a segment of sciatic nerve approximately 2 cm. long was removed from goats and dogs. The gap in the nerve was filled with small, frozen, dried, irradiated nerves and the entire area was wrapped with a Millipore cuff. Functional progress was recorded with motion pictures and conduction across the area determined electrophysiologically. These investigators have found that functional return was noted in three of the four dogs and in two goats. The transmission of the nerve impulses across the graft was found in three dogs and in one goat.

Further attempts toward studying regeneration in the spinal cord of cats and monkeys in which an attempt to facilitate such regeneration by spinal fusion and inhibition of vertebral mobility, has been undertaken by Dr. Feringa. Successful fusion after the spinal cord was transected, has also been done on 13 monkeys. The effect of these procedures on spinal cord regeneration has not as yet been evaluated.

Dr. Feringa has also initiated a study as to the possible role of auto-immune antigens in cerebral scar formation with a hypothesis that the glial scar which blocks axonal regeneration following central nervous system injury may result from an auto-immune response to the release of brain-antigen following the injury provoked breakdown of the blood-brain-barrier.

Dr. Feringa is also using tritiated-labelled thymidine to determine in spinal cord regeneration whether such axons which are regenerating come from pre-existing neurons or from outgrowth of neuroblasts that have newly differentiated from ependymal cells. In this study, he has injected the tritiated thymidine in newts approximately 12 days after cord transection and has noted labelling of the ependymal cells only. This would indicate that whatever regeneration was present in the newt spinal cord was derived from new neurons in response to spinal cord transection.

Two other projects, one related to the neural pathways of color vision in the fish and one related to gustatory function following heterogeneous reinnervation of the taste buds in the fish, have been initiated by Dr. Bernstein.

THE INTERCONNECTIONS OF THE VESTIBULAR AND AUDITORY SYSTEMS

Under the guidance of Dr. Grant Rasmussen, the investigation in connection to the vestibular and auditory system has continued at all levels, from the peripheral receptor to the cortex. In this study, advantage was taken of the histochemical method of Koelle in which acetylcholinesterase histochemical stains were utilized since efferent fibers possess a much higher degree of this activity than do afferent fibers. With such stains, Dr. Rasmussen and his colleagues have been able to follow efferent fibers of the vestibular and cochlear nerves from the brain to the epithelium of all the receptor organs of the inner ear in the chinchilla and the cat. In the organ of Corti, the efferents were easily traceable to the inner and outer hair cells. Those fibers to the outer hair cells have been observed to bend sharply apicalwards winding in and out the rows of receptor cells. Dr. Rasmussen points out that each fiber could, therefore, make synaptic contact with numerous hair cells, but this remains to be proven by electron microscopic studies of the organ of Corti. He points out, however, that all these fibers are directly related to the efferent bundle, originating in the brain, since all traces of acetylcholinesterase activity disappears seven to ten days after cutting the bundle in the medulla.

Dr. Sanford Palay points out that Dr. C. Smith of St. Louis, has for several years, been studying with the electron microscope, the innervation of the organ of Corti in the normal guinea pig. Such studies thus far show disproportionately large numbers of efferent type endings in respect to the relatively few afferent fibers.

Drs. Rasmussen and Richard Gacek have previously demonstrated the efferent innervation of all the vestibular receptors, as well as the auditory, with the aid of fiber degeneration methods. And, Professor Gosta Dohlman, a visiting scientist from Lund, Sweden, in Dr. Rasmussen's Laboratory, has

demonstrated a high concentration of acetylcholinesterase in some fibers located next to the hair cells of these receptor organs. Once again, the histochemical reaction disappears in all vestibular receptors following transection of the efferent bundle in the medulla. Professor Dohlman and Dr. Rasmussen have found, contrary to previous evidence, that not all of the ascending fibers from the colliculus pass in the brachium to the medial geniculate body. Nor do all such fibers enter the geniculate body or terminate in all parts of it. Many fibers leave the brachium of the inferior colliculus to ascend in the reticular formation of both sides to undetermined destinations. Other fibers course outside of the principal auditory pathway and terminate in the subthalamic nucleus. Most surprising to these investigators was the finding that the auditory fibers of the brachium, which is the principal auditory pathway to the geniculate body, apparently terminate only in the inferior portion of the geniculate body. The dorsal portion of the geniculate body remains strikingly free of fiber and synaptic degeneration following destruction of the inferior colliculus. It would appear, therefore, that the dorsal portion of the medial geniculate body may have an entirely different function.

Dr. Donald Morest is undertaking a study for this particular problem using modified Nauta-Gygax methods for determination of axonal and preterminal degeneration, subsequent to brain lesions in the thalamus and midbrain. These will be combined with synaptic stains, such as those of Glees and Rasmussen. The cellular morphology will be worked out by suitable Golgi methods. Doctors Rasmussen and Walther are studying the ascending and descending connections between the medial geniculate body and the auditory cortex, by placing small lesions in various locations of the medial geniculate body and the auditory cortical areas AI, AII, and EP, the insular and temporal gyri. The resultant fiber and synaptic degeneration is studied by these investigators by Marchi stains, and by the Nauta and Rasmussen synapse preparations. Geniculotemporal projection studies to the auditory cortex are still not complete but the cortical feedback connections of the geniculate body have been completed. It has been demonstrated that the principal part of the medial geniculate body, except for its dorsal portion, receives descending connections from auditory areas AI, AII, and the posterior ectosylvian gyrus (EP), and also from the more recently established auditory areas located in the insular and temporal cortex. A large cell portion of the medial geniculate body which is usually considered as a non-auditory cell group, also receives connections from the classical cortical auditory areas. In addition, such areas project to other auditory nuclei at lower levels of the brainstem, i.e, the nucleus of the inferior colliculus of both sides and the dorsal nucleus of the lateral lemniscus of the same side.

Further studies on the efferent component of the vestibular nerves are being carried out by Doctors Gacek and Rasmussen to

determine the manner in which such fibers terminate to locate the exact cells of origin of the efferent vestibular fibers. Techniques used are the Sudan Black technique, previously described by Dr. Rasmussen for the study of Wallerian degeneration of nerves of the petrous bone, combined with serial sections of the vestibular root and all of its peripheral branches, including the receptor epithelium. In addition, Protargol silver, Glees' ammoniacal silver, and the Nauta-Gygax axonal degeneration methods are employed. The method of Brodal based upon the phenomenon of retrograde cell changes is used to demonstrate the cell bodies of the efferent fibers. Elucidation of the ultimate termination of such efferent fibers is necessary to understand how such an efferent system may regulate sensory input as the cochlear efferent bundle does to the cochlea.

CONCLUSION

This then concludes the Annual Report of the Intramural Program of the National Institute of Neurological Diseases and Blindness. A variety of studies related to basic and applied mechanisms of neural action have enriched the Institute's understanding of the functions of the nervous system. Basic studies may outlive the more readily (and hence, more easily outmoded) applied investigations. However, the interchange of scientific knowledge among all investigators , whether such knowledge has immediate application or derived for the sake of knowledge itself has nevertheless acted to make a stronger unit and a more critical one. The scientists at NIH and laboratories throughout the world have collaborated to make such studies possible. This Institute would like to acknowledge such joint studies:

National Naval Medical Institute	(Biophysics)
Physiology Laboratories at Cambridge and Plymouth, England	(Biophysics)
Computer Laboratories of National Bureau of Standards	(Biophysics)
Walter Reed Medical Center	(Ophthalmology)
Department of Biophysics, Purdue, University	(Biophysics)
Brookhaven National Laboratory	(Ophthalmology)
Oak Ridge National Laboratory	(Medical Neurology) (Surgical Neurology)
Donner Laboratory, Berkley, California	(Surgical Neurology)
Biophysics Department, Uppsala, Sweden	(Biophysics)
Fort Knox Microwave Laboratory	(Surgical Neurology)
Departments of Surgery of Boston University Columbia University	(Neuroanatomical Sciences)

Armed Forces Institute of Pathology	(Surgical Neurology)
National Naval Medical Center	(Surgical Neurology)
Max Planck Institute, Germany	(Neurochemistry)
Marine Biological Laboratories	(Biophysics)
Woods Hole, Massachusetts	(Neurophysiology)
Technishe Hochschule, Darmstadt	(Biophysics)
National Institute of Arthritis and Metabolic Diseases	(Medical Neurology)
	(Neurophysiology)
National Heart Institute	(Medical Neurology)
	(Pharmacology)
National Institute of Allergy and Infectious Diseases	(Ophthalmology)
National Cancer Institute	(Medical Neurology)
National Institute of Mental Health	(Neurophysiology)
	(Neurochemistry)
Physiology Department, University of Texas	(Neurophysiology)
Walter Reed General Hospital	(Surgical Neurology)
U. S. Agricultural Experimental Station, Beltsville, Maryland	(Neurochemistry)

Many of these investigations would have been impossible without such free interchange of scientific information and collaborative research with such units.

The Clinical Director has indicated his appreciation to the Nursing Staff of the Institute, in particular, Miss Margaret Hulburt and her three Chief Nurses, Mrs. Thompson, Miss Soltow and Miss Maccia. The Institute is also indebted to the Office of the Director of the Clinical Center and to the various clinical service units of this Center, in particular, Diagnostic Radiology, Clinical Pathology, and Anatomic Pathology.

G. Milton Shy, M.D.

Report of the Clinical Director

(Calendar Year 1960)

Since the last report, 808 patients were admitted to the Clinical Center by the National Institute of Neurological Diseases and Blindness. The average patient stay was 35.3 days in a total of 23,257 patient days. 1009 patients were examined in the Admissions and Followup Unit, while 1612 patients were seen in consultation at the request of other Institutes.

These patients cooperated in studies on glaucoma, cataract, toxoplasmosis, retinal diseases, macroglobulinemia, color vision defects, tearing, neuromuscular disorders, cerebral neoplasia, basic (cerebral) metabolic abnormalities, epilepsy, involuntary movements, pain, cerebral metastases, and psychoses.

On behalf of the National Institute of Neurological Diseases and Blindness, the Clinical Director gratefully acknowledges the support of the Director and Staff of the Clinical Center. Without this invaluable support, our work would be impossible. The Neurology Nursing Service and the Neurological Operating Room Staff deserve special mention for their dedication and excellence.

BRANCH OF ELECTROENCEPHALOGRAPHY AND CLINICAL NEUROPHYSIOLOGY

SUMMARY OF 1960 ACTIVITIES

1. Routine diagnostic service.

A considerable portion of the Branch activity was dedicated to routine diagnostic service. This has been true in the past, but never before to the extent of this year. In the twelve month period included between the last report (prepared November 1, 1959) and the present one (prepared November 1, 1960), the load of electroencephalographic examinations has largely exceeded those of any single previous year (see figures below). While it is true that many of such examinations were an intrinsic part of several research projects of this Branch, a large number of them was carried out purely for diagnostic purposes or, rather often, in an effort to provide cooperation (on a simply consultation-technical basis) to research projects originating from other Branches of our Institute or from other Institutes.

A total of 1844 EEG examinations was performed with the following distribution among patients of the different Institutes:

NCI.	365
NHI.	94
NIAMD. . . .	114
NIMH	8
NMI	-
NINDB. . . .	1263
Total	1844

It is interesting to compare these figures with those of the previous six years as given below:

	1954	1955	1956	1957	1958	1959
NCI . . .	46	123	322	370	306	291
NHI . . .	29	30	44	30	76	48
NIAMD. . .	23	29	38	68	81	53
NIMH . . .	7	2	31	38	27	104
NMI . . .	36	27	41	52	56	73
NINDB. . .	644	1065	995	999	956	1100
Total	785	1276	1471	1557	1502	1579*

(*for eleven months)

The record monthly average number of examinations of last year (143.5) has been largely exceeded this year (153). The majority of referrals is still from our Institute but, as in the last four years, about one-third of examinations were requested by other Institutes, and in particular, by the National Cancer Institute which accounts for over 60% of the referrals outside of NINDB.

These figures are provided not simply for their statistical interest but to show the steady growth in this particular activity of the Branch. If one compares the available monthly averages of EEG examinations carried out in large hospital services or leading Neurological Centers throughout the Nation one sees that our figures would fall in the upper half. While this situation reflects a growing confidence in the practical usefulness of this diagnostic method, and while it might be looked upon favorably from a point of view of providing training facilities, it is important to take into consideration that in most other EEG laboratories full time reporters are often two or even three and that their main, if not exclusive, task is actually to supply routine diagnostic service.

It is obvious that if EEG examinations are requested, it is because they are considered a necessary or at least useful method of investigation and the Branch should make every effort to cooperate. On the other hand, it should be equally evident that such "cooperation" implies the almost full time activity of the (professionally) under-staffed Branch. This problem is simply brought to the attention of the Associate Director for his consideration.

1. Research activity.

Notwithstanding the above-described load of routine diagnostic service, it has been possible for the Branch to carry on a certain amount of independent research both in the clinical and in the experimental fields.

a) Clinical. In this field four projects have been undertaken and two are now considered completed. Project 1c is a major one which is partially a continuation of similar projects described in previous Annual Reports. In its broad lines it deals with several aspects of epilepsy with emphasis on focal seizures. Essentially its main purposes are the understanding of the pathophysiological mechanisms of seizures and their diagnosis. It is a project which is carried out, in part, in close cooperation with the NINDB Branch of Neurological Surgery. Details are available in the attached description. Due to the rather general nature of this problem and to its numerous aspects, different ways of approach have been employed so that the project itself could actually be broken down into various individual studies. Some of these have been of a purely critical-theoretical nature. Specifically, an attempt was made to collect and clarify our present concepts on what characterizes the behavior of epileptic neuronal pools and on what distinguishes it from that of normal neural aggregates. This critical review, based on both

personal and other people's data, was presented at a Symposium and will be published in the near future.

Another similar approach was used to analyze the problem of human focal epilepsy. In this study the various findings both clinical and electroencephalographic, are objectively discussed in an effort to determine the validity of our present concepts of this particular neurological entity and, indirectly, of the more general concepts of cortical functional localization. This study, too, will be presented, upon invitation, at a Symposium sponsored by the American Epilepsy Society.

We would like to add that while on the one hand the two preceding essays can hardly be classified as "research projects", it would be unfair, on the other, to consider them merely as literary efforts of questionable practical significance. It is our impression that in the complex and intriguing field of convulsive disorders there is a justified need for some abstract, critical thinking and to put in their right perspectives the innumerable findings and observations which keep accumulating.

Partly because of these considerations, the elaboration of the material (clinical and electroencephalographic, including 32 electrocorticograms and the results of deep electrography carried out on 15 patients)has been slowed down in the past year and no major findings or conclusions are yet available. These should be expected, however, for the next year. Only an analysis of ictal episodes in patients with temporal lobe epilepsy could be completed. In it, special attention was paid to the relationships between vegetative symptomatology and electrographic changes. The results are presently in press.

A second, smaller project 2(c) was completed. This, too, deals indirectly with epilepsy but its main emphasis is on possible pathophysiological mechanisms of that characteristic EEG pattern commonly associated with "petit mal" seizures. Two cases with available pathological findings were analyzed and it was possible to conclude that a purely coincidental association of this EEG pattern (3/sec spike-and-wave complexes) and the type of pathology (neoplasm) was extremely unlikely. In addition, because of the particular topography of the lesions (in these as well as in other similar cases found in the literature) it was felt that a primary or exclusive cortical role in the "genesis" of this electrographic entity was rather improbable.

A third project 3(c) also completed and in press, deals with the electroencephalographic diagnosis of secondary tumors of the brain. This study correlates number and size of metastatic nodules, particularly cortical and intracerebral, with the original EEG findings in a series of 93 patients in which a complete post-mortem was available. Among other data it shows a quasi-absolute relationship between presence of local slow abnormalities in the record and size of the lesion(s); the critical dimension appears to be 2 cm, larger lesions being always, and smaller ones almost never, detectable in the EEG. Similar relations were found

to exist between size of the metastasis and presence or absence of neurological signs.

A fourth project 4(c) deals with the general problem of spontaneous and induced electrical activity of various subcortical structures in humans using the technique of (chronic) depth electrography. This project differs from analogous ones described in previous reports in that it utilizes non-epileptic subjects and in the fact that it plans to investigate less the activity of pararhinal structures and more that of thalamus and basal ganglia. A wealth of findings has already been collected but their analysis and elaboration has just begun.

Major conclusions should become available in the first part of next year. Specific details and different aspects of this research project are found in the attached description.

b) Animal experimentation. In this field there have been two new projects all the others described in preceding Reports having been completed and published. Both investigations were carried out with the very active collaboration of Dr. A. Morillo from Bogota, Columbia.

The first project 5(c) deals with cortical control of sensory input, and specifically it concerns the central visual system. It was carried out on cats and it is closely related to, and actually a continuation of, project 41C of 1959. In this previous study it was possible to demonstrate the existence of cortical influences upon incoming messages at the level of the lateral geniculate nucleus. These influences were likely the result of corticifugal (orthodromic) phenomena, however, it had not been possible to rule out in a crucial way, the possible role of antidromic effects. The experimental design of the present project (see description) allows this critical differentiation and the obtained results confirm the existence of cortico-geniculate fibers as well as their modulatory influences upon the activity of geniculate elements. The project is practically completed and the various data should be ready for publication in the near future.

The second project 6(c) is still in course, having been initiated a few months prior to the preparation of this report. It deals with the functional morphology of the "callosal" response in sensory cortical areas of the cat and with the relationships between this potential and those evoked by peripheral stimulation. The experimental findings related to this study should be completed by the year's end and their analysis and elaboration should be ready in the first part of 1961.

3. Other activities.

Besides diagnostic service and research, the Branch provides facilities for training in electroencephalography. This activity takes advantage of the very useful collaboration of the technical personnel but it also implies certain duties on the part of the Chief of Branch

with an inevitable sacrifice of time taken from more productive activities. While this has obvious shortcomings, we are convinced of the usefulness of carrying on this program as long as possible. The Nation has a serious shortage of properly trained electroencephalographers as shown by a recent survey of the American EEG Society. Our Branch, being one of the not too numerous recognized training centers, has the moral obligation of assisting, within the limits of its possibilities, the efforts of the Society in this task.

In the past year, four people, Drs. W. Lewis, M. Lane, R. Gumnit and F. Merritt, have spent a period of 6 to 12 months, for more or less formal training, in clinical electroencephalography. There was in addition a Research Associate in neurophysiology (Dr. A. Morillo).

The Branch Chief was officially invited to participate in three different Symposia (see project description) and in an additional two which will be organized late in 1961 on the occasion of the International Congresses of Rome and Washington. He has also become an official member of the examining Board in Electroencephalography and has been invited to participate as a lecturer in the "advance course in EEG" to be held in Marseille, France, just prior to the Rome meeting. Starting from next January he will become the Managing Editor for America and Far East of The Journal of Electroencephalography and Clinical Neurophysiology, an international journal which is the official organ of the Federation of the various EEG Societies throughout the world.

4. List of publications.

This list includes only papers related to projects described in preceding Reports but published in 1960. It excludes abstracts, papers still in press and those - either in press or published - related to current projects. The latter are found in Part B of the respective Project description sections.

1) Ajmone Marsan, C. and Abraham, K. "A Seizure Atlas". EEG Clin. Neurophysiol. Suppl. #15, 1960, pp 215.

2) Widen, L. and Ajmone Marsan, C. Unitary analysis of the response elicited in the visual cortex of cat. Arch. ital. Psiol. 1960, 98: 248-274.

3) Mirsky, A.F., Primac, D.W., Ajmone Marsan, C., Rosvold, H.E. and Stevens, J.R. A comparison of the psychological test performance of patients with focal and non-focal epilepsy. Exper. Neurology, 1960, 2: 75-89

4) Widen, L. and Ajmone Marsan, C. Effects of corticipetal and corticifugal impulses upon single elements of the dorsolateral geniculate nucleus. Exper. Neurology, 1960, 2: 468-502.

5) Gerin, P. Microelectrode investigations on the mechanisms of the electrically induced epileptiform seizure ("after-discharge"). Arch. ital. Biol. 1960, 98: 21-40.

Serial No. NINDB-1(c)
1. Electroencephalography & Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. Continuation of 79C, 81C, (1956), 79C, 84C (1957), 2C, 3C (1958), 37C, 38C, (1959)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the problem of epilepsy with emphasis on focal seizures.

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: John Van Buren, Maitland Baldwin

Cooperating Units: NINDB - Branch of Neurological Surgery

Man Years (calendar year 1960):
Total:
Professional:
Other:

Project Description:

Objectives: This is a major long range project which has, among its main objectives: 1) understanding of the pathophysiological substrate of convulsive disorders, 2) improving or refining the diagnostic criteria in different types of epilepsy, 3) analyzing clinico-electrographic correlations in ictal episodes.

Some aspects of this project have already been described in detail in preceding reports and some might actually be considered completed, having appeared in final published form (see Summary) or having been presented at Medical meetings in this current year.

Patient material and methods: Subjects of this study are the epileptics admitted by the cooperating unit (NINDB. Branch of Neurological Surgery). Several methods are used for the different aspects of the project under investigation:

Part B included Yes [x] No [ ]

a) extensive, serial routine EEGs during wakefulness, drowsiness and sleep.

b) direct recording of electrical activity from the exposed cortex during surgery.

c) direct recording from cortical and subcortical structures in patients with chronically implanted electrodes (using CRO and inkwriting apparatuses).

d) elicitation of seizures in the three abovementioned situations, with systemic chemical or local repetitive electrical stimulation.

e) critical analysis and discussion of clinical and, chiefly, experimental data, both personal or collected from the literature.

Major findings: Part of the project related to clinico-electrographic correlations is completed. The results were outlined in last year's project 36C and have recently appeared in a published monograph (see Summary).

Other similar studies, emphasizing the relationship between autonomic-vegetative and subjective signs and EEG changes were presented at the February meeting of the Eastern Association of Electroencephalographers.

In 22 ictal episodes observed in patients with temporal lobe epilepsy, autonomic recording gave evidence of a remarkably stereotyped response consisting predominantly of hypertension, tachycardia, fall of skin resistance, esophageal peristalsis, inhibition of gastric motility, and inhibition of respiration. Variation in this pattern lay predominantly in the failure of a given change to appear or in the sequence of the changes. In nearly all instances, however, failure of the patient to respond was preceded by some alteration in autonomic function.

Most of the aurae were reported when no significant electrographic activity had occurred and many autonomic changes appeared without an electrographic correlate. No vegetative change was seen to accompany the onset of focal ictal activation either on the surface or in the depth of the temporal lobe, although such changes might appear later as the activity incireased in extent and amplitude. The bilaterally symmetrical roughly synchronous bursts of activity usually of greater amplitude in the frontal regions might

or might not be accompanied by autonomic changes. No change was seen in the level of responsiveness with these brief bursts. Sudden generalized loss of voltage or "flattening" of the record formed a constant warning of imminent clinical seizure activity either as an automatism or a generalized convulsion. Most of the changes in the level of response or of autonomic function appeared against a background of gradually increasing generalized epileptic activation with or without a preponderance in the temporal region. Under these circumstances, various phenomena including loss of responsiveness occurred without evident correlation in the electrographic record.

Discussion and critical survey of the properties characterizing the epileptic neuronal pool were part of the same broad project. These theoretical considerations have been presented in June at a Symposium on "Epileptic mechanisms" sponsored by the American EEG Society. In this presentation the morphological features characterizing the electrical activity of epileptic neuronal aggregates (ENA) as recorded by means of macro and microelectrodes were briefly reviewed. Some basic neurophysiological phenomena were surveyed and, by comparing their role in the behavior of normal neural pools and of ENA, the most likely differential characters in the two situations were outlined. Basic mechanisms possibly involved in generation and maintenance of epileptiform activity were analyzed and discussed. In the activity of ENA, two aspects were distinguished: inter-ictal and ictal. In analyzing the processes responsible for the paroxysmal cyclical firing (inter-ictal), and for the organized, repetitive, self-sustained (ictal) activity in ENA and for the transition from one to the other, it was felt that more than simply quantitative differences characterize these two types of activities; It was suggested that while the former can theoretically take place in the isolated neuron, the latter can only occur in ENA. The significance of specific nervous elements and/or the role of particular portions of the cell in any given ENA was de-emphasized. The full paper is presently in press.

A similar, theoretical-analytical study deals with another aspect of this same project in the field of human epilepsy. It is specifically concerned with the problem of focal epilepsy and the validity of the classical concepts which are currently accepted in this particular form of seizures. A detailed analysis of clinical and electroencephalographic data upon which such concepts are based and a critical discussion of those which might lend themselves to different interpretations or which suggest the necessity of a revision of thinking are the object of a paper to be presented in the near future at a Symposium on Focal Epilepsy sponsored by the American Epilepsy Society.

For the problem of temporal lobe epilepsy, electroencephalographic and electrocorticographic data, as well as those obtained from patients with chronically implanted electrodes are in the process of being analyzed and correlated with various clinical findings as part of a major project which is described in previous reports as well as in the current yearly report of the Branch of Neurological Surgery (see).

Significance to the program of the Institute: This project, in its main lines, is a continuation of other, closely related projects whose significance has been repeatedly emphasized in previous reports of the last six years.

Proposed course of project: Future plans have been mentioned above in the section of Major Findings. Essentially they consist of collecting and elaborating the various findings. This task is, of necessity, slowed down by the wealth of data which are steadily accumulating, the unavoidable delay of securing sufficient post-operative follow-up periods (of at least 2-3 years) in a large number of subjects, and, particularly, by the material impossibility of dedicating enough time to the project itself because of the routine service and other research activity for which this modestly staffed Branch is responsible.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Ajmone Marsan, C. Electrographic aspects of "epileptic neuronal aggregates. Epilepsia, 1961, in press.

Van Buren, J. and Ajmone Marsan, C. A correlation of vegetative and EEG components in temporal lobe epilepsy. J. Neurosurgery, in press.

(See also Summary of the Branch activities)

Serial No. NINDB-2(c)
1. Electroencephalography & Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Pathologic findings in patients with "centrencephalic" electroencephalographic patterns.

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: William Lewis

Cooperating Units: None

Man Years (calendar year 1960):
Total:
Professional:
Other:

Project Description:

Objectives: This is not a systematic research project but simply a case analysis and presentation. Although it deals indirectly with problems similar to those outlined in the previous project (36c), it is described separately because its emphasis is on cryptogenic epilepsy or, more correctly, on that particular electrographic pattern which is considered characteristic of this form of seizures. The title of the project is self-explanatory.

Patient material and methods: Analysis of clinical, pathological and electroencephalographic findings in two patients (one from the NCI and the other from the Branch of Neurological Surgery, NINDB). Survey of the literature and statistical treatment of data pertaining to incidence of brain tumors and of EEG patterns characterized by 3/sec spike and wave complexes.

Major findings: These were presented at the meeting of the Pan American Medical Association in Mexico City in May. They are summarized here in their final, published form:

Part B included Yes [x] No [ ]

1. The reasons for disagreement and controversial interpretations related to pathogenesis and anatomotopographic "origin" of the bilaterally synchronous 3-per-second wave and spike complexes are outlined and briefly discussed.

2. Clinical, pathologic, and electrographic findings in two patients are presented. In both, the electroencephalogram shows an almost typical and, in any case, highly suggestive pattern of "petit mal" epilepsy; one patient had a large metastasis of one cerebellar hemisphere but no clinical evidence of seizures, and the other had petit mal, temporal lobe episodes, and generalized convulsions, together with a large astrocytoma demonstrated within the right temporal lobe extending deeply toward the thalamus.

3. All similar cases of brain tumors associated with bilateral 3-per-second wave and spike complexes reported in the literature are reviewed. They are so rare that the question of a coincidental association of 2 unrelated phenomena might be raised; however, statistical evaluation shows that, if chance alone were operating, one would expect such an association in 48.2 of 1,000,000 cases. Thus, in 3 published reports of cases totaling 1,071 brain tumors, the "expected" frequency of cases with 3-per-second wave and spikes would be 0.0517 case, while the actual "obtained" frequency is 6 cases. This discrepancy is highly significant($Chi^2 = 600$), and one can safely conclude that the two phenomena are indeed related.

4. The large predominance of subtentorial or midline localizations in tumors accompanied by this characteristic electroencephalographic pattern is suggestive of a primary brain stem role in the mechanism of production of the pattern itself.

Significance to the program of the Institute: Same as for previous project.

Proposed course of the project: This study is considered completed and has already appeared in print - (see Part B).

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Ajmone Marsan, C. and Lewis, W. R. Pathologic findings in patients with "centrencephalic" electroencephalographic patterns. Neurology, 1960, 10: 922-930.

Serial No. NINDB-3(c)
1. Electroencephalography & Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Pathological and electroencephalographic analysis of metastatic tumors of the brain.

Principal Investigator: Raymond Strang and Cosimo Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total:
Professional:
Other:

Project Description:

Objectives: Although the value of EEG in diagnosis of primary brain tumors is well established, less definite information exists regarding the efficacy of the method in confirming or revealing cerebral metastases. In such cases, the diagnostic problems are often quite different and size and multiplicity of metastatic lesions add to the complexities of EEG analysis. This project is primarily concerned with these latter aspects of the problem.

Patient material and methods: The clinical records of all patients with intracranial metastases who were admitted and had autopsy at the National Institutes of Health between 1953 and early 1959 were examined and 93 cases having concomitant EEG examination were selected for preliminary study. Presence or absence of focal delta activity in their records was noted and the number and size of metastatic lesions was established as accurately as possible.

Major findings: These have been described in detail in a paper. Summary and conclusions, as they will appear in the published form, are reported here:

This report deals with a correlative analysis of pathological-topographical and electroencephalographic findings in 93 patients

Part B included Yes [x] No [ ]

with intracranial metastases, all verified at autopsy. Difficulties associated with clinical and EEG diagnosis of metastatic lesions as opposed to primary brain tumors as well as the relative usefulness of several routine EEG procedures in this diagnostic field are briefly discussed.

In the case of intracerebral lesions a high degree of correlation was found between EEG and neurological findings: few (3) neurologically silent metastases were detected by EEG and, conversely, very few of those with positive neurological signs were missed by EEG examination.

By using rather strict and uniform electrographic criteria, the most significant factor for correct EEG (as well as clinical) localization and identification appears to be the absolute size of the metastatic lesion. A diameter of 2 cms represents the borderline size for a lesion to be detected both by neurologic and by EEG examination.

In 44.5% of 52 cases with single or multiple intracerebral metastases a correct EEG localization was obtained. Eighty one per cent of all lesions measuring more than 2 cms and only one out of the numerous lesions smaller than 2 cms were correctly detected by EEG. In analogy, it was exceptional for metastases less than 2 cms in diameter to manifest themselves by neurologic symptoms or signs.

Problems related to, and results of EEG identification of brain stem (13 cases) and/or cerebellar (29 cases) lesions have been briefly mentioned. Among other known factors, the frequent association of multiple supratentorial metastases would appear to be the main complicating one.

Significance to the program of the Institute: This project is of some practical interest to the programs of both our Institute and of the National Cancer Institute. Its main significance is in the diagnostic and possibly prognostic fields in those cases in which neurological surgery is considered. It provides some useful clinico-electrographic criteria while re-emphasizing - on the basis of objectively documented data - some of the limitations of electroencephalography. Even in its negative aspects, however, it provides indirect information of practical significance.

Proposed course of the project: The project originally started in 1959 but not mentioned in last year's Report, is now completed. The paper has been submitted and accepted for publication.

Serial No. NINDB-3(c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Strang, R. and Ajmone Marsan, C. Pathological and electroencephalographic analysis of metastatic tumors of the brain. A.M.A. Arch. of Neurol., in press.

Serial No. NINDB-4(c)
1. Electroencephalography & Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Depth electroencephalography

Principal Investigators: John Van Buren and Cosimo Ajmone Marsan

Other Investigators: None

Cooperating Unit: Branch of Neurological Surgery, NINDB

Man Years (calendar year 1960):
Total:
Professional:
Other:

Project Description:

Objectives: This is part of a major project ("Studies on involuntary movements") described in detail in the report of the Branch of Neurological Surgery NINDB. The objectives of this specific aspect of the project are the study of electrical activity, both spontaneous and induced, in various subcortical structures of man. In previous years, similar studies had been carried out but these were exclusively limited to rhinencephalic formations and were chiefly related to epileptic disorders of the so-called temporal lobe type (see reports 81C(1956), 79C(1957), 2C(1958), 37C(1959)).

Patient materials and methods: See report of Branch of Neurological Surgery.

Major findings: Data have been collected on only 9 patients and still need to be analyzed, thus no conclusive findings can be reported to date. Most of the chronically implanted electrodes (from 14 up to 42 in each subject) have been placed within the thalamus and basal ganglia or in close proximity to these structures, as well as extradurally. These placements permit a direct, simultaneous recording of the electrical activity and a correlation among the rhythms at

Part B included Yes ☐ No ☒

these different levels. Of particular interest appears this correlative study during various phases of sleep. The technique of local stimulation with brief electrical pulses and of CRO recording of the evoked potentials have provided promising preliminary results which might be usefully applied to the determination of preferential functional inter-relationship between various structures in the human brain. Finally, the analysis of EEG changes before and after destruction of certain subcortical nuclei is likely to yield interesting findings which might be indirectly useful in the routine EEG diagnosis as well as in clarifying the possible role of these nuclei in the genesis or functional substrate of the so-called "spontaneous activity" of the cortex.

Significance to the program of the Institute: This project is of particular interest for this Branch for self-evident reasons and is closely related to the major project on "Involuntary Movements" of the Branch of Neurological Surgery NINDB.

Proposed course of the project: Collecting more data on a larger number of subjects and analyzing the already available findings.

Serial No. NINDB-5(c)
1. Electroencephalography & Clin. Neurophysiology
2. Neurophysiology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cortical control of sensory imput

Principal Investigators: Cosimo Ajmone Marsan and Arturo Morillo

Other Investigators: None

Cooperating units: None

Man Years (calendar year 1960):
Total:
Professional:
Other:

Project Description:

Objectives: This project is closely related to a similar study (see outline in 41C 1959) which was completed and has recently been published. Its main purpose is to confirm the existence of cortico-thalamic influences and, specifically, to rule out the possible role of antidromic phenomena which was discussed but not definitively eliminated in the preceding paper.

Methods employed: Technical details were described in projects 5C (1958) and 41 (1959). Acute experiments were carried out on 24 "cerveau isolé" cats. Tungsten microelectrodes, stereotaxically oriented, were used to record unitary potentials from the dorsal lateral geniculate nucleus. These were activated chiefly by single flashes of light and by electrical stimulation of the optic tract, both types being used as "test" stimuli. The response, elicitable in the ipsilateral visual cortex by callosal impulses generated by electrical stimulation of the contralateral homologous cortex, was used as "physiological" conditioning stimulus.

Part B included Yes ☐ No ☒

Major findings: These are still in the process of being analyzed in detail and no definite conclusions are available at the moment this report is prepared. It appears, however, that:

1) effects are demonstrable at the level of the lateral geniculate nucleus following stimulation of the contralateral visual cortex;

2) these efferent (cortico-geniculate) effects are very likely related to the callosal response and consist of, a) activation of unitary elements of the lateral geniculate body, or b) modification in the pattern of their firing when the latter is elicited by test photic or optic tract stimulation;

3) this conditioning influence may be inhibitory or facilitatory and can be shown for relatively short as well as for fairly long intervals between conditioning and test stimuli;

4) in view of the specific experimental design it is safe to conclude that these effects are not dependent upon antidromic phenomena.

Significance to the program of the Institute: This research deals with the general problem of perception and is related to integrative mechanisms at a cortico-thalamic level. It is primarily a project of physiological interest but its close relationship to the puzzling problems of vigilant consciousness, attention or "specific arousal" offers suggestive implications of obvious clinical interest.

Proposed course of the project: The project is considered completed as far as the experimental part is concerned. The various findings are presently in the process of being analyzed and the final results should be available for publication in the near future.

Serial No. NINDB-6(c)
1. Electroencephalography & Clin. Neurophysiology
2. Neurophysiology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Analysis of callosal responses in the visual cortex of cat.

Principal Investigator: Arturo Morillo

Other Investigators: Cosimo Ajmone Marsan

Cooperating units: None

Man Years (calendar year 1960):
Total:
Professional:
Other:

Project Description:

Objectives: This project is somewhat related to the previous one (5c) and to projects described in 1958 (5C) and 1959 (39C). Its main purpose is to study: a) the morphology of the "slow" electrical potential evoked in the visual cortex by stimulation of the contralateral homologous region; b) the temporal distribution of unit activity similarly elicited and its relationship to the slow response; c) the inter-relationships between gross and unitary callosal phenomena and the "visual" response elicitable in the same cortical area by photic or optic tract stimulation.

Methods employed: Acute experiments are performed on "cerveau isole" cats. Recording by means of tungsten microelectrodes and of conventional surface electrodes; standard stimulation techniques as already described in previous similar projects.

Major findings: Experiments are still in course and the data obtained from the first 15 animals up to the time of this report, are still to be analyzed.

Part B included Yes ☐ No ☒

Significance to the program of the Institute: Besides that discussed in the preceding, related project (5c) this present study os of special interest for the Branch because of its close connection with the problem of inter-hemispheral synergism and bilateral synchrony of electrical cortical rhythms in human electroencephalography.

Proposed course of the project: Complete the series of experiments and analyze data.

MEDICAL NEUROLOGY BRANCH

SUMMARY, 1960

See Pages 4 - 17 of the Scientific Director's Summary

Serial No. NINDB-7(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Histopathological and Chemical Investigations of Neuromuscular Disorders

Principal Investigator: G. Milton Shy

Other Investigators: Donald Silberberg
Theodor Wanko
Daniel Drachman
Robert Krooth

Cooperating Units: None

Man Years:
Total: 8.0
Professional: 6.0
Other: 2.0

Project Description:

Objectives:

Again this is a continuation of a long term study on diseases effecting skeletal muscle in man. This study is now of such magnitude that new portions as activated are being turned over to other investigators within the Institute as follows:

Enzyme Histochemistry and tissue culture - Dr. King Engel and Dr. Franz Seitelberger

Ultra-structure - Dr. Theodor Wanko

Contractile Proteins - Dr. Beni Horvath and Dr. Karl G. Henriksson

Thus Dr. Franz Seitelberger and Dr. Theodor Wanko have completed a thorough study on "Central Core" disease

and may be found in their reports. Dr. Engel's projected interest may be seen in his report.

The present studies now are concentrated about the understanding of carcinomatous myopathy, and the application of immunopathological studies to disease of muscle generally, in addition to the studies listed in the previous report.

Methods Employed:

Approximately 250 cases of varying types of muscle disease are now admitted annually to this Institute. All such cases receive thorough pathological and electrophysiological studies. Special studies are carried out where indicated as noted in results.

In addition, studies utilizing both animal and human material are continuing in the study of muscle regeneration utilizing autoradiography with H^3 thymidine. Chromosomal studies are being done particularly in myositis ossificans and dystrophia myotonica. These are being done with Dr. Robert Krooth utilizing tissue culture techniques. The final studies in familial periodic paralysis utilizing intracellular recordings, chemical analysis of cations, tracer techniques, endocrine and metabolic studies, histochemistry and pharmacologic methods have been completed.

Proposed Course of Project:

As noted, primary attention now will be placed on initiation of new projects listed above, i.e., immunopathological techniques and a thorough study of carcinomatous myopathy using all parameters such as were used in familial periodic paralysis.

Major Findings:

1. In familial periodic paralysis

   (1) Muscle potassium and sodium are not significantly elevated in familial periodic paralysis during attacks.

   (2) There is no significant change in the resting potential of the paralyzed fibers.

   (3) During attacks the membrane of the fiber does not conduct a propagated potential when stimulated directly.

   (4) Hyperpolarization apparently cannot be indicated as the cause of paresis.

(5) Variations in potassium exchanged across the muscle membrane do not allow a constant equilibrium to be recorded between muscle and serum.

(6) Attacks of paresis may occur without hypokalemia.

(7) An abnormality of glucose tolerance is reconfirmed in these patients.

(8) Triamcinolene causes an exacerbation of the disease.

(9) Glucagon and epinephrine induce weakness in the resting patient.

(10) Oliguria with positive potassium and sodium balance was a constant feature of such attacks of paresis in these patients.

(11) An absence of secondary hyperglycemia responses in one such patient upon repeated injections of glucagon may be attributed to an impairment in hepatic glycogen synthesis or reduced output of lactate and pyruvate from resting muscle. A review of the literature makes the latter assumption unlikely.

(12) Aldosterone excretion is not increased during attacks, as measured by the double isotope derivative method.

(13) D-l-aldosterone administration does not precipitate attacks.

(14) No significant increase in blood volume (which could trigger aldosterone release) was recorded during attacks.

(15) Administration of pitressin tannate does not precipitate attacks.

(16) Thus it is unlikely that an abnormality of aldosterone metabolism is responsible for such attacks of paresis.

(17) Excess sodium intake or administration of 9a-fluoro-hydrocortisone will precipitate attacks and make the patient weaker.

(18) Low sodium intake or administration of 3-(3-oxo-17-hydroxy-19-nor-4-androstan-17-) proprionic acid lactone (an aldosterone antagonist) will aid in prevention of attacks.

(19) The effect of sodium ingestion is still unexplained.

(20) An elevated basal metabolic rate has no adverse effect on the patient.

(21) A fall in the basal metabolic rate is accompanied by an exacerbation of the patient's symptoms.

(22) Such an effect seems to be independent of TSH secretion.

(23) During attacks there is an ingress of intracellular water, as seen in the light and electron microscopes, and measured chemically.

(24) This is manifest in the light microscope by loculated vacuoles, many of which contain PAS positive staining granules.

(25) The loculation suggests that such fluid is membrane enclosed.

(26) This is confirmed with observations by electron microscopy, in which such vacuoles appear to arise in the sarcoplasmic reticulum oriented parallel to longitudinal axis of the myofibrils.

(27) A characteristic change in the mitochondria is also suggested by the electron microscopic findings.

(28) The granules seen in the light microscope are confirmed in the electron microscope.

(29) It is suggested that such granules may represent the accumulation of non-diffusible intermediates in glycogen anabolism, possibly in the form of non-diffusible anionic sub-polymerized glycogen molecules, which would cause an influx of electrolytes and water into muscle to maintain osmotic, electrical, and Donnan equilibria.

2. In H^3 thymidine, labelling only of the sarcolemmal nuclei in tissue culture of embryonic muscle has been accomplished. No labelling has occurred in perfused human biopsy material which has shown the characteristic pathologic etiology associated with regeneration. This could be because the mitotic interval exceeds the profusion time or that mitotic division cannot occur in adult striated muscle. To clarify this, chronic (3 mo.) osmotic infusion of tritiated thymidine has been started in the rat.

3. The study of carcinogenic myopathies has been just initiated with no results as yet.

4. An ancillary finding in this study has been a group of dysproteinemic myopathies associated with Sjogren's syndrome which show a typical myopathic picture clinically with gamma globulins up to 10 gm/100 cc, and plasma cell infiltrations into the muscle.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Shy, G.M., Wanko, T., Engel, A., and Rowley, P.: Studies in Familial Periodic Paralysis. J. Exper. Neuro. (In Press).

Honors and Awards relating to this project:

None

Serial No. NINDB-8(c)

1. Medical Neurology Branch
2. Section on Neurological Disorders Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The Physiology and Pharmacology in Myasthenia Gravis

Principal Investigator: Dr. G. Milton Shy

Other Investigators: Dr. Richard Irwin
Dr. Charles Payne

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	1.5
Other:	2.0

Project Description:

Objectives: (1) The testing of new anti-cholinesterase compounds in patients with myasthenia gravis. (2) Accuracy of electromyography in neuromuscular disorders.

Methods Employed: (1) At the present time, toxicity studies have been finished upon the carbamate form of deoxy-demethyl-lycoramine hydrochloride and it has now been tested in a series of eight myasthenic patients. This is a quaternary ammonium compound and hence may be administered only intravenously. The initial tests have indicated that the therapeutic level of this particular drug is in the order of four micrograms/kilogram. There appears to be in initial studies, a much wider safety factor between the therapeutic level and the toxic level of the drug. The duration of action of this compound appears equal to that of pyridostigmine. If continued studies on this compound demonstrate equal effectiveness, then the tertiary compound will be synthesized and toxicity studies carried out so that oral medication may be available of the lycoramine compound. This latter phase is largely carried out by Dr. Richard Irwin. (2) Electrophysiological studies of muscle abnormalities have continued in which measurement of duration of action potentials of different motor units of different muscles in normal and abnormal patients are continued, nerve conduction times are computed and nerve stimulation is carried out for abnormal fatigue states, such as sarcoid carcinomatous myopathy and myasthenia gravis. Approximately 343 such studies were carried out in the past year.

Major Findings: Sterile pryogen-free anti-cholinesterase compounds of the lycoramine group are now available for intravenous administration, and have been administered to eight patients with promising results. This study will continue using many more patients in the coming year. Electromyographic studies have demonstrated an accuracy of approximately 89% when compared to the clinical, and 92% when compared to the pathological studies of patients with neuromuscular disease. These studies are now being summarized in the form of a handbook to be used for standardized electromyography within this Institute.

Significance to the Program: Utilization of more potent anti-cholinesterase substances, which also may be produced more cheaply than those now marketed, has a large implication in the treatment of myasthenia gravis. The electromyogram when utilized with pathological control and with a knowledge of physiology of the motor unit, has proven to be an extremely valid diagnostic tool in the study of neuromuscular disorders.

Proposed Course of the Project: Both these are long-term projects and will continue for the duration of admission of patients with neuromuscular disorders.

Part B Included yes / / No /X/

Serial No. NINDB-9(c)

1. Medical Neurology Branch
2. Section of Neurological Disorders Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The Use of Monoamine Oxidase Inhibitor as an Anti-convulsant Medication for Centrencephalic Seizures

Principal Investigators: Bushnell Smith, M.D.
Darwin Prockop, M.D.

Man Years (calendar year 1960):
Total: 1.0
Professional: 1.0
Other: 0

Patient Days (calendar year 1960): 250

Project Description:

Objectives: The objective of the present study was to evaluate the effectiveness of a monoamine oxidase inhibitor as an anti-convulsant in persons with centrencephalic seizures. The methods utilized were those described in the previous annual report, NINDB-34(c) (59), except that Nialamide (N-/2-) benzylcarbamyl) ethylamino 7-isonicotinamide was employed as the monoamine oxidase inhibitor. The subjects, wherever possible, were given a constant number of tablets which were either placebos or Nialamide. Thus, the amount of Nialamide could be varied by the investigator without the knowledge of the patient. Fall in blood pressure and marked suppression of sweating were employed clinically as signs of effective dosage. Tryptamine determinations were done on the urine at various dosage levels.

Major Findings: The therapeutic effectiveness of the monoamine oxidase inhibitor as an anticonvulsant was not substantiated during this report. However, a side finding in this study may be of paramount importance in patients suffering from centrencephalic seizures. Thus, in two patients receiving monoamine oxidase inhibitor, clonus, hyperreflexia, and confusion occurred. When small amounts of Tryptophane were given by mouth in one patient, the same findings occurred, after the inhibitor had been stopped for two weeks and Tryptophane was again given by mouth.

Tryptophane normally does not produce these findings. Thus, such patients appear to act as though they already had some type of monoamine oxidase inhibitor within their system. Also as an alternative, however, the lesion might lie at the level of 5-hydroxytryptamine and be due to deficiency in decarboxylase. This part of the investigation is still being continued at this time. Larger numbers of patients with centrencephalic seizures will be utilized to determine if all respond in this way to monoamine oxidase inhibitor. Patients with other types of seizures will also be utilized as controls.

Significance to Neurological Research: (1) It would appear that the monoamine oxidase inhibitors have slight, if any, control of centrencephalic seizures. (2) There may be some indication that patients with centrencephalic seizures handle Tryptophane differently than patients that do not have such a disorder.

Proposed Course of Project: To study more patients for such side effects and to determine if the lesion lie at the decarboxylase or monoamine oxidase level.

Part B Included Yes / / No /X/

Serial No. NINDB-10(c)
1. Medical Neurology Branch
2.
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Cytological Studies of Neuromuscular Phenomena in Patients with Neuromuscular Disease and in Experimental Animals.

Principal Investigator: W. King Engel, M.D.

Other Investigators: Genevieve A. Drews
Marco Mumenthaler, M.D.

Cooperating Units:

Man Years:
Total: 4.0
Professional: 3.0
Other: 1.0

Project Description:

I. Objective: To study cytological phenomena of the neuromuscular system through the disciplines of histochemistry and tissue culture. Studies on two groups, (a) Patients with Neuromuscular Disease and (b) Experimental Animals, will be mutually complementary.

II. Methods Employed:

1. Enzyme histochemistry of skeletal muscle.

(a) Patients - Biopsies of normal and abnormal muscle will be studied for the distribution and amount of several known enzymes (enzymes in the oxidative and glycogenic pathways; enzymes of the neuromuscular junction).

(b) Experimental animals - The distribution of several enzymes (vide supra) will be studied before, during, and after innervation of the developing muscle, as well as following denervation of the mature muscle cell

(c) Patients and experimental animals - New histochemical methods for significant enzymatic and protein constituents of the normal muscle cell will be developed.

2. Tissue culture of skeletal muscle.

(a) Patients -

(i) Methods will be established for good reproducible growth of adult human muscle in culture.

(ii) Normal and abnormal human muscle obtained by biopsy will be studied in regard to its growth and metabolic needs in culture.

(iii) The histochemical aspects (vide supra) of normal and abnormal human muscle growing in culture will be studied.

(b) Experimental animals -

(i) Certain aspects of the nerve cell-muscle cell relationship will be established and tested pharmacologically and histochemically in culture.

(ii) Previous studies of the cytochemical localization of constituents in the cultured muscle cell will be extended, especially in regard to the enzyme systems mentioned above.

(iii) Response of the glucose-glycogen system to certain pharmacologic agents will be studied in the cultured muscle cells.

(iv) Previous electrophysiological and immunological studies of cultured muscle cells may or may not be extended in the near future.

III. Patient Material: Skeletal muscle biopsies are usually taken for diagnostic purposed from in-patients with neuromuscular disease. At the time of biopsy, an additional piece of muscle tissue is removed for the histochemical and tissue culture studies indicated above.

IV. Major Findings: This report is being written in the third month of setting up a new laboratory. Major findings of earlier work have been included in reports from another laboratory (Clinical Neuropathology, Dr. I. Klatzo).

V. Significance to bio-medical research and the program of the Institute: There are reports of the histochemical distribution of several enzymes in normal (Dubowitz and Pearse, 1960) and abnormal (Dubowitz and Pearse, 1961) human skeletal muscle. Similar and additional studies will be done and on muscle biopsy material from our patients with neuromuscular disease. Abnormalities found will be correlated with possible pathophysiologic mechanisms of the disease in an attempt to arrive at the etiology or at steps in the pathogenesis. Also of probable direct clinical significance will be studies of the reaction of the glucose-glycogen system in cultured skeletal muscle in response to certain pharmacologic agents; this is of interest because an abnormality of carbohydrate metabolism has been suspected in several neuromuscular diseases (e.g., Familial Periodic Paralysis, Shy et al., 1961; McArdle's Disease, Schmid and Mahler, 1959).

It has been reported (Geiger and Garvin, 1957) that skeletal muscle from patients with muscular dystrophy shows poor growth in tissue culture compared with muscle from normal persons. After methods have been established for good reproducible growth of adult human muscle, the previous study will be repeated and expanded especially in relation to the growth, metabolic needs, and histochemistry of abnormal and normal muscle.

In the experimental animal, information of basic significance about the nerve cell-muscle cell relationship will be obtained. Especially, the histochemical distribution of a number of enzymes will be studied before, during and after innervation of the developing muscle as well as following denervation of the mature muscle cell. In addition, an attempt will be made to establish a nerve cell-muscle cell relationship in tissue culture. When the relationship is established, the pharmacologic response and histochemistry will be studied. In regard to cultures of muscle cells only, previous studies of the cytochemical localization of cellular constituents will be extended. Basic information obtained regarding the normal nerve cell-muscle cell relationship and the characteristics of the muscle cell will be correlated with information about abnormalities in neuromuscular diseases in an attempt to clarify and understand the abnormalities in the latter.

VI. Proposed Course of the Project: As this report is being written at the inception of a new project, the proposed course of that project is included under "Methods Employed".

Part B included Yes / / No /X/

Serial No. NINDB-11(c)
1. Medical Neurology Branch
2. Section on Biophysical Applications
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Native State of Bacterial and Viral Desoxyribonucleic Acid (DNA)

Principal Investigator: Donald J. Cummings, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 1.0
Other: None

Project Description:

Objectives: To determine the in vivo molecular weight of bacterial and viral DNA. The best documented evidence on gene size has been found with the E. coli-bacteriophage system. The result obtained must be interpreted with foreknowledge of the size of the entire genome, i.e., the molecular weight of the DNA. While basically simple, earlier attempts to measure this quantity have been failures due to major difficulties in handling this extremely long, fragile molecule. The proposed project is designed to measure the native molecular weight of DNA and consequently give a quantitative estimate of the gene size.

Methods Employed: Standard microbiological procedures for growth and purification of E-coli bacteria and T-even bacteriophages.

DNA will be separated from other biological material in as "normal" a fashion as possible. With regard to the virus, the genetic material will be extruded from the tail core in the same manner as the virus actually injects it into host cells. E-coli DNA will be obtained by utilizing F^+ and F^- cells and

causing the F^+ cells to mate with cell wall material from F^- cells, thus liberating the DNA from the F^+, leaving the rest of the cell intact. The extracted DNA will then be studied in the Spinco Model E ultracentrifuge using the cesium chloride density gradient method. The molecular weight of this native DNA can be determined from the density of the DNA and the shape of the flotation band; i.e., the narrower the band, the higher the molecular weight.

Significance to the Program of the Institute: As is well known, many neurological disorders are transmitted genetically. In any genetic phenomenon, it is important to know the gene size so that any molecular changes observed as part of the abnormality can be related to the genome. This new estimate of the gene size can be used in interpreting the genetic findings in some neurological abnormalities.

Proposed Course of the Project: To extend the molecular weight determination to DNA from other organisms in an effort to correlate genome size with the complexity of the organism.

Part B included No

Serial No. NINDB-12(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Electrolytes and Energy Metabolism in Normal and Epileptogenic Cerebral Cortex *in Vitro*.

Principal Investigator: Dr. D. B. Tower

Other Investigators: Dr. G. M. McKhann, Dr. B. Smith, Mr. E. L. Peters.

Cooperating Units: Dr. T. Wanko, NINDB Section on Ophthalmological Cytology; Dr. M. Baldwin, NINDB Surgical Neurology Branch.

Man Years (calendar year 1960):
Total: 1.3
Professional: 0.9
Other: 0.4

Project Description:

Objectives: To study the *in vitro* metabolism of electrolytes (K^+, Na^+, Cl^-, etc.) and of energy-producing cycles and components thereof in incubated slices of cerebral cortex from experimental animals and from human patients operated on for focal epilepsy.

Methods: See previous reports.

Patient Material: Obtained from NINDB patients admitted for other purposes.

Major Findings:

A. Effects of Exogenous NH_4^+ on Electrolytes and Fluid Spaces:

(1) When cerebral cortex or subcortical white matter slices were incubated with 5 - 10 mM NH_4Cl added to the incubation medium, NH_4^+ ions were found to compete with K^+ for inward transport into slice cells according to the following relationship:

$$U = C \left([NH_4^+]_o / [K^+]_o \right)^{\underline{c}}$$

where U = uptake of free NH_4^+ in μEq./ml. non-chloride space; $[NH_4^+]_o$ and $[K^+]_o$ = medium concentrations of ammonium and potassium ions respectively in μEq./ml.; C = a constant typical for the experimental conditions = 19.9; and c = the regression coefficient = + 0.48 ($\pm$ 0.045). This relationship holds at least for external NH_4^+ concentrations of 5 - 10 μEq./ml. and external K^+ of 5 to 30 μEq./ml. The free NH_4^+ space within incubated slices under these conditions exceeded 100%, clearly indicating concentration of NH_4^+ ions within the cells against a concentration gradient, i.e. "active" inward transport. An equivalence of intracellular K^+ was "displaced" by the NH_4^+ (e.g. with external NH_4^+ = 9 and K^+ = 27 μEq./ml, the free NH_4^+ concentration in slice non-chloride space = 19 μEq./ml.). No effect of NH_4^+ on outward transport of Na^+ was observed. These effects were equally present in neurons and glia, as judged by comparable behavior of subcortical white matter slices (after correction for lipid-bound NH_4^+).

(2) Concurrently the non-chloride space of neurons, but not of glial cells (as deduced from comparisons of subcortical white with cortical slices), decreased significantly when cortical slices were incubated with 5 - 10 μEq./ml. NH_4^+ in the medium - the average value being only 80% of normal controls. As reported in another project [see 60 - NINDB - 13(c)], a significant fraction of the total NH_4^+ transported into neuronal cells is "bound" as amide N of free and protein-bound glutamine. The extra glutamine so formed is produced by amidation of glutamic acid and hence involves an equivalent loss of "fixed" intracellular anions:

$$Glu - COO^- + NH_4^+ \rightarrow Glu - CONH_2 + H_2O$$

(Acid—anion) $\rightarrow$ (Amide - no charge)

Consequent to this loss of intracellular anion, a decrease of fluid volume (extrusion of H_2O) would be expected and is consistent with the decrease of neuronal non-chloride space observed. This interpretation is supported by the fact that no change from control values of neuronal non-chloride space occurred when cortical slices from methionine sulfoximine - treated animals were incubated with added NH_4^+. Under these conditions neuronal synthesis of free and "extra" protein-bound glutamine is inhibited, but inward transport of free NH_4^+ is unaffected. Thus, one mechanism for neuronal regulation of intracellular anion and fluid balance appears to reside in the free and protein-bound glutamine system.

B. Effects of Exogenous NH_4^+ on Oxidative Metabolism:

(1) Under the same conditions, the oxygen consumption of both

cortical and subcortical white matter slices was significantly depressed (average - 16%). In additions cortical slices, but not subcortical white matter slices, exhibited a doubling of aerobic glycolysis (lactic acid production) and an equivalent elevation of glucose consumption. The known greater glycolytic capacity of neurons is thus emphasized by these conditions. Samples from methionine sulfoximine - treated animals behaved in identical fashion, indicating that these effects of exogenous NH_4^+ ions are distinct from effects on the glutamine synthesizing systems.

(2) Comparable studies on cerebral cortex mitochondria demonstrated similar effects of NH_4^+. When pyruvate or α-ketoglutarate were substrates, oxygen consumption and substrate utilization in the presence of 15 mM NH_4Cl were decreased 30 - 40% from control values, but no change from control values was observed when succinate, glutamic acid or γ-aminobutyric acid were substrates. Hence the NH_4^+ effect was selective for pyruvate and α-ketoglutarate oxidation. Normal oxidation of succinate and normal P/O ratios in all cases seemed to rule out any direct effect of NH_4^+ on the cytochrome system. Lack of effect on glutamic acid or γ-aminobutyric acid oxidation indicated that the metabolic pathway of the latter remained intact (note that mitochondria possess no glutamine synthesizing enzymes). And no evidence for disturbance of DPNH oxidation in slices or mitochondria was obtained. Consequently the effect of NH_4^+ under these conditions appears to be a direct interference with the oxidative decarboxylation of pyruvate and α-ketoglutarate, both of which utilize similar mechanisms and cofactors. The exact nature of the block remains to be elucidated.

C. Morphological Correlates by Electron Microscopy:

A pilot study in collaboration with Dr. Wanko has been initiated with two objectives: (1) To provide morphological control of subcellular fractionation procedures aimed at obtaining uncontaminated fractions of mitochondria, microsomes, etc. so that the metabolic characteristics of these fractions can be more precisely evaluated. So far this aspect has concentrated on mitochondria with an attempt to satisfy the criteria of morphological purity and of maintenance of the biochemical characteristics of crude mitochondrial preparations intact. Preliminary success has been achieved by recentrifugations at various centrifugal forces to remove cell debris and myelin fragments but some microsomal contamination still remains. (2) To study the effects of NH_4^+ and other agents which affect the fluid space distribution of incubated slices on the morphological characteristics of these slices. Identical pairs of slices (control and test slice) are incubated for 1 hr. at 37° C in bicarbonate-saline-glucose in our specially modified Warburg vessels fitted with a wide-mouth sealable port. At the end of incubation the slices are

fixed *in situ* with OsO_4, floated out through the unsealed port in the fixative and continued for electron microscopy. Preliminary data indicate that by comparison with control slices, those incubated with 10 mM NH_4Cl exhibit greater astrocytic swelling (? larger chloride space), swelling of endoplasmic reticulum lumina and possibly loss of the fibrillar components of astrocytes.

Significance of Project: Energy metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism, which is clearly dependent upon it, provides an important link between cellular chemistry and the functional activity of impulse conduction. The understanding of the factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged function in hyperactivity states such as seizures.

Proposed Course: To continue studies suggested by the findings cited here.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications:

1. Tower, D. B.

   Neurochemistry of Epilepsy: Seizure Mechanisms and Management. Springfield, Thomas, 1960.

2. Tower, D. B.

   Some neurochemical aspects of cortical neurobiology. In Tower, D. B. and Schadé, J. P. (Eds.) Structure and Function of the Cerebral Cortex. Amsterdam, Elsevier, 1960, p. 411.

3. Tower, D. B., Wherrett, J. R. and McKhann, G. M.

   Functional implications of metabolic compartmentation in the central nervous system: Interrelationships of cellular proteins and amino acids, fluid spaces and electrolytes, and oxidative metabolism, as delineated by ammonia. In Kety, S. S. (Ed.) Regional Chemistry of the Nervous System. Oxford, Pergamon, in press.

4. McKhann, G. M. and Tower, D. B.

   Ammonia toxicity and cerebral oxidative metabolism. Amer. J. Physiol. in press.

5. McKhann, G. M. and Tower, D. B.

   A modified manometric vessel for special studies on tissue metabolism. Anal. Biochem. in press.

Honors and Awards: None.

Serial No. NINDB-13(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Free and Protein-Bound Amino Acids in Normal and Epileptogenic Cerebral Tissues.

Principal Investigator: Dr. D. B. Tower

Other Investigators: Dr. G. M. McKhann, Dr. J. R. Wherrett, Mr. E. L. Peters

Cooperating Units: Dr. R. W. Albers, NINDB Lab. Of Neuroanatomical Sciences
Dr. G. M. Shy, NINDB Medical Neurology Branch
Clinical Center Pharmacy
Dr. H. Waelsch, New York State Psychiatric Institute
Dr. M. Baldwin, NINDB Surgical Neurology Branch

Man Years (calendar year 1960):
Total: 2.9
Professional: 1.5
Other: 1.4

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids of the glutamate and aspartate groups in the free and protein-bound pools of cerebral tissues from experimental animals and from human patients operated on for focal epilepsy, with emphasis on cerebral cortex studied in vitro.

Methods: See previous reports and under Major Findings.

Patient Material: From NINDB patients admitted for other purposes, except for case discussed under D (below) admitted specifically for this study.

Major Findings:

A. γ-Aminobutyric Acid Metabolism:

(1) The finding that in vitro at least 40% of the total substrate oxidized by the Krebs cycle in cerebral cortex passes via the γ-aminobutyric acid pathway [cf. 59-NINDB-3(c)] has been confirmed in vivo in collaboration with Dr. Albers. Brains of mice were

sampled at varying intervals after injection of pyruvate-3-C^{14} systemically and the specific activities (cpm/μM) of brain glutamic acid and γ-aminobutyric acid determined. In less than 2 minutes after injection the S.A. of the latter exceeded that of its immediate precursor, glutamic acid. This finding indicated that there is metabolic compartmentation of the reaction sequence: pyruvate$\rightarrow$glutamic acid$\rightarrow\gamma$-aminobutyric acid, i.e. the total tissue pools have not equilibrated at this time. Hence no quantitative calculation of flux rates is possible. Nevertheless, the data clearly indicate that a major portion of substrate oxidation is proceeding via the γ-aminobutyric acid pathway <u>in vivo</u>.

(2) Factors regulating the channelling of substrates via either the γ-aminobutyric acid or succinyl-CoA pathways were investigated in detail, utilizing cat cerebral cortex mitochondria incubated with γ-aminobutyric acid-1-C^{14} with simultaneous measurement of oxygen consumption and $C^{14}O_2$ evolution (as a measure of γ-aminobutyric acid utilization) in a specially designed Warburg flask. [A standard single side-arm vessel was modified by substitution of a round-bottom center-well to permit complete quantitative removal of viscous, organic alkalis (e.g. Hyamine) used to absorb the CO_2, and by addition of a wide-mouth port in the vessel wall which could be sealed by rubber cap and aluminum sealer, so that injections into or withdrawals from the closed vessel could be made by needle puncture of the rubber cap.] The following conclusions may be drawn from these studies:

(a) A reciprocal relationship exists between γ-aminobutyric acid utilization and α-ketoglutarate concentration present, such that as concentration of the latter was increased, utilization of the former decreased. In the presence of arsenite (an inhibitor of α-ketoglutarate oxidative decarboxylation) γ-aminobutyric acid utilization was enhanced and was not depressed by high α-ketoglutarate concentrations.

(b) The reciprocal relationship could be modified by altering mitochondrial permeability (pre-incubation in hypotonic sucrose). With such "swollen" mitochondria depression of γ-aminobutyric acid utilization by high concentrations of α-ketoglutarate was significantly less than in "intact" mitochondria. And at lower concentrations of α-ketoglutarate, the addition of oxidized DPN to such "swollen" mitochondria markedly enhanced γ-aminobutyric acid utilization. This latter effect was not seen in "intact" mitochondria (which are essentially impermeable to exogenous DPN) nor at high α-ketoglutarate concentrations in either mitochondrial preparation.

(c) It is concluded that there is a reciprocal regulation of

the fraction of total substrate metabolized via each of the two parallel pathways between the α-ketoglutarate to succinate stage of the Krebs cycle (i.e. via γ-aminobutyric acid or via succinyl CoA) and that the availability of oxidized DPN may be an important factor in this regulation.

B. Free and Protein-Bound Glutamate and Aspartate Metabolism:

(1) A reproducible procedure for isolation, enzymatic hydrolysis, and assay of liberated amino acid residues of the glutamate and aspartate group of cerebral proteins has been developed. Total mixed proteins from the TCA-insoluble residue of brain samples are isolated after extraction of nucleic acids and lipids and hydrolyzed enzymatically by Viokase (a whole dessicated pancreas preparation). The hydrolysate is then assayed enzymatically for total glutamate (glutamic acid + glutamine), for glutamine and for aspartic acid (by Meister's Cl. perfringens method) and for asparagine (by Meister's guinea pig serum asparaginase). Free amino acids in the tissue samples remain in the TCA-soluble fraction and can be assayed simultaneously by the same methods. Where samples have been incubated with C^{14}-labelled amino acids, the isolated protein is redissolved and reprecipitated to constant specific activity (cpm/mg. protein) before hydrolysis, and the amino acids of the free pool and protein hydrolysate are separated chromatographically for counting of C^{14}-activity by gas flow and liquid scintillation counters. Where samples were incubated with N^{15}-labelled NH_4Cl, the various ammonia fractions liberated enzymatically (or by acid) from constituent amino acids of the free and protein pools are collected by the Conway microdiffusion technique, and aliquots assayed spectrophotometrically. N^{15} is determined by the procedure of Sprinson and Rittenberg, using a Consolidated Electrodynamics Corp. Model 611 Mass Spectrometer.

(2) Data for normal cat brain samples may be summarized as follows:

(a) Dry protein residues from 36 samples of cerebral cortex averaged 8.4 (± 0.5)% of the original tissue wet weight.

(b) Total amide N was estimated to be 56 μM/100 mg. dry protein with 80 to 90% attributable to glutaminyl plus asparaginyl residues therein.

(c) Total glutamate (glutamic acid + glutamine) was 78.05 (± 1.35) μM/100 mg. dry protein. Of the total 44.1 (±2.2)% was glutamine in cortical samples immobilized immediately after brain excision, whereas in slices prepared for incubation and in those incubated 1 hr. at 37° C. in bicarbonate-saline-glucose glutamine represented 36.8 (± 1.8)% and 37.6 (±1.55)%

respectively of the total. These values differ significantly ($P<0.001$) from that obtained on immediately immobilized samples. Since total glutamate did not change, it is concluded that during slice preparation, there was deamidation of protein glutamine to protein glutamic acid with the liberated amide NH_3 appearing free in the tissue. This is consistent with a number of studies which have postulated protein glutamine to be a major source of endogenous cerebral NH_3.

(d) Total aspartate (aspartic acid + asparagine) was 58.9 (±1.4) μM/100 mg. dry protein. Of the total 45 to 50% was asparagine under all three of the foregoing conditions of sampling. No evidence for comparable lability of the protein asparagine amide group was obtained.

(3) When cortical slices were incubated with C^{14}-labelled glutamic acid or glutamine, rapid labelling of free pool amino acids was found, with specific activities (cpm/μM) comparable to those previously reported from this laboratory [Cf. 58-NINDB-27(c)]. The S.A. of protein-bound glutamic acid and protein-bound glutamine were 0.1% or less of the respective free pool S.A. after 1 hr. incubation. This extent of incorporation of labelled amino acids into cerebral proteins *in vitro* is of the same order of magnitude as that found *in vivo* by Waelsch after comparable periods of brain exposure to injected, labelled amino acids.

(4) Cortical slices incubated in the presence of 40 mM malonate (which is associated with abnormally high levels of free pool glutamic acid [Cf. 59-NINDB-1(c)]) exhibited a decrease of protein-bound glutamine from control values of 38.3 (±0.95)% of total protein glutamate to 33.3 (±2.6)%. The difference is of borderline significance ($P = 0.02$), but may reflect transfer of additional protein glutamine amide NH_3 to support the additional accumulation of free glutamic acid from α-ketoglutarate under these conditions.

(5) When cortical slices were incubated in the presence of 10 mM NH_4Cl (which is associated both with abnormally high levels of free pool glutamine [Cf. 58-NINDB-27(c)] and also with intracellular concentration of free NH_4^+ ions [Cf. 60-NINDB-12(c)] there was a significant increase of protein glutamine at the expense of protein glutamic acid - from control values of 36.7 (±1.9)% of total protein glutamate to 50.45 (±3.7)%, the difference being significant ($P<0.001$). No effect on protein aspartate was observed. And no such effect was seen in proteins isolated from incubated slices of subcortical white matter, suggesting that the NH_4Cl effect is neuronal and not glial in locus. Finally the NH_4Cl effect was abolished in incubated slices of cortex from animals intoxicated with methionine sulfoximine (which completely inhibits free pool glutamine synthesis [Cf. 58-NINDB-27(c)]),

suggesting that the NH_4Cl effect on cortical proteins is mediated via free glutamine, i.e. a transamidation from free pool glutamine to protein-bound glutamic acid. Results of studies using N^{15}-labelled NH_4Cl (99 Atoms % excess N^{15}) are consistent with this last interpretation. In these studies the N^{15} Atoms % excess in incubated cortical slice fractions were: free NH_3 81, free glutamine amide N 40, protein-bound glutamine amide N 7. Thus the N^{15} Atoms % excess of amide N from protein-bound glutamine is 18% of that for free pool glutamine, whereas the C^{14} specific activity of protein glutamine was less than 0.1% of free pool glutamine S.A., thus clearly ruling out direct incorporation of the entire free glutamine moiety into protein. Comparable in vivo studies, where 10 mM/kg NH_4Cl was injected parenterally 45 min. prior to brain excision, indicate similar changes in cortical protein glutamine, but data are not yet complete. Altogether these findings clearly support the concept that the amide group of protein-bound glutamine is labile and imply important relationships to endogenous nitrogen storage, transfers and reactions (as e.g. with biologically active amines) as well as to the intracellular "fixed" anion pool.

C. Protein Purification and Fractionation

(1) The studies cited above represent work with the total mixed proteins extractable from cerebral tissues. Eventually more detailed studies on isolated protein fractions will be essential to an elucidation of the metabolic characteristics. For this purpose work has continued on a model protein, guinea pig serum asparaginase [Cf. 59-NINDB-10(c)], chosen because of its availability and easily assayable enzymatic activity, which has been used to check the maintenance of protein integrity throughout numerous manipulations. Partial purification by Na_2SO_4 and Ca-phosphate gel fractionation was previously shown to increase the specific activity of the enzyme (S.A. = activity/mg. protein N) about 9-fold. This preparation was found to contain 3 to 5 visible components by electrophoresis and ultracentrifugation. Now we have achieved what appears to be complete purification by chromatographing the partially purified preparation on a DEAE-cellulose column, according to the gradient elution procedure of Sober and Peterson. The elution pattern shows 5 major fractions at the following buffer values and effluent volumes: I (0.005 M phosphate pH 7.0, 0.08 liters), II (0.02 M phosphate pH 5.9, 0.6 liters), III (0.02 M phosphate pH 5.9 + 0.1 M NaCl, 0.75 liters), IV (same buffer, 0.9 liters), and V (0.02 M phosphate pH 5.9 + 0.5 M NaCl, 1.1 liters). None of these carry asparaginase activity. The enzyme is concentrated in a minor fraction between components III and IV at 0.8 liters effluent volume and represents only about 1% of the total protein nitrogen applied. The specific activity of the separated enzyme is over 2000 μM/hr. per mg. protein N or a total purification of approximately 220-fold.

(2) The very low protein concentration has prevented the usual studies of purity by electrophoresis and ultracentrifugation for the relatively small quantities so far purified. Comparisons of the properties of the purified enzyme with those of the unchromatographed crude preparation indicate no essential differences in terms of pH dependence (optimum 9.6), Michaelis constant ($Km \approx 2.2 \times 10^{-3}$), effects of various common inhibitors and activators of deamidases, and stability in the buffered, frozen state.

(3) In addition to the foregoing approach, the utilization of cellular fractionationation by differential centrifugation (alone or in combination with subsequent column chromatography) is being developed, and preliminary data on cerebral cortex proteins present in various subcellular fractions is being obtained. At present it appears that the percentage of total glutamate attributable to glutamine is considerably higher in microsomal protein than in that from other fractions.

D. Cerebrospinal Fluid γ-Globulins in a Case of Friedreich's Ataxia with Hypogammaglobulinemia:

(1) Another facet of cerebral proteins concerns interrelationships of cerebral tissue proteins with those of the CSF and of both of these with serum proteins, especially in neuropathological states. In collaboration with Dr. Shy this interesting case was studied from this point of view. A number of cases of Friedreich's Ataxia studied at NINDB have shown elevated CSF γ-globulins, so that this case with the additional feature of very low to absent serum γ-globulins presented a unique opportunity.

(2) Initial studies of serum and CSF proteins by paper electrophoresis demonstrated that while on bimonthly injections of γ-globulin (10 ml. i.m.), serum γ-globulin was ~3.1% of the total protein (normal average = 16.6%) and CSF γ-globulin was 23.6% of the total (normal average = 10.0). However quantitatively the serum was calculated to contain 186 mg/100 ml. compared to CSF content of 5.5 mg/100 ml. Thus it was felt that no conclusion could be drawn regarding the altered percentages.

(3) After stopping γ-globulin therapy for 6 months, the patient was re-examined and found to have serum γ-globulins ~ 4.4% (273 mg/100 ml.) and CSF γ-globulins ~ 2.5% (0.8 mg/100 ml.). He was then injected with 40 μC of I^{131}-labelled γ-globulin (prepared by the Clinical Center Pharmacy) and subsequent activity of serum and CSF γ-globulins followed at frequent intervals. In both cases the total activity of the sample was compared with electrophoretic fraction activities and in the case of CSF

with activities of the total sample after dialysis. Findings may be summarized as follows:

(a) The labelled serum γ-globulin decreased after injection with a biological half-life of about 13.5 days (corrected for I^{131} decay), but the plot suggested two components - one with a half-life of about 4.5 days and the other of about 45 days. A wide range (from 5 to over 30 days) has been reported for the biological half-life of γ-globulin injected into normals and hypogammaglobulinemic patients. If, as suggested by our data, more than one disposal process is operating, these reported variations become understandable. The significance of the two decay rates is not clear, but it may be that the more rapid reflects tissue distribution and sequestration, while the slower reflects true catabolism.

(b) There was definite passage of labelled γ-globulins into the CSF, the peak activity occurring about 7 days after injections Subsequently CSF activity declined at a rate roughly parallel to the serum decline, although it was our impression that the CSF rate was slower, suggesting some degree of sequestration in the CSF compartment (estimated biological half-life about 25 days). Because of uncertainties about the exact concentrations of γ-globulinin serum and CSF at the low levels encountered here, it is impossible to express the activities as specific activities (cpm/mg. γ-globulins) with any degree of certainty. Consequently it is not certain whether the labelled CSF γ-globulin ever reached equilibrium with labelled serum γ-globulins. When expressed as cpm/mg. <u>total</u> protein, the S.A. of CSF γ-globulins never exceeded about one-half the serum S.A. Similarly compared to the normal average CSF to serum γ-globulin ratio (on a concentration basis) of .0.234%, the activities ratio (cpm/ml.) of CSF to serum here never exceeded 0.17%. (These findings may be contrasted with Fishman's data for I^{131}-labelled albumin injected into normal dogs, where CSF activity was evident within 1 hr. and the equilibrium time for CSF and serum S.A. (cpm/mg. <u>total</u> protein) was about 20 hours, with serum half-life of 6.3 days and CSF half-life of 8.0 days).

(4) This study represents an isolated and pathological situation, but it does carry certain implications with respect to the relationships of serum γ-globulins to those in the central nervous system compartment in diseases such as multiple sclerosis, CNS syphilis, Friedreich's ataxia, and multiple myeloma where elevated CSF γ-globulins commonly occur.

<u>Significance of Project</u>: This project is of fundamental importance in helping to delineate some of the interrelationships between cerebral structure (proteins) and metabolism and between metabolism and functional activity, so that an eventual understanding of the integration of these facets of cellular neurochemistry can be realized.

Proposed Course: Emphasis will be placed on studies of free and protein-bound aspartate metabolism, utilizing micromethods now in development, on survey of species similarities or variations of protein glutamate and aspartate characteristics, on subcellular fractionation for possible localization studies, and on the characteristics of epileptogenic brain samples in these respects.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications:

1. Tower, D. B.
Neurochemistry of Epilepsy: Seizure Mechanisms and Management. Springfield, Thomas, 1960.

2. Tower, D. B.
The neurochemistry of asparagine and glutamine. In Brady, R. O. and Tower, D. B. (Eds.) Neurochemistry of Nucleotides and Amino Acids. New York, Wiley, 1960, p. 173.

3. McKhann, G. M., Albers, R. W., Sokoloff, L., Mickelsen, O. and Tower, D. B.
The quantitative significance of the gamma-aminobutyric acid pathway in cerebral oxidative metabolism. In Roberts, E. (Ed.) Inhibition in the Nervous System and Gamma-Aminobutyric Acid. Oxford, Pergamon, 1960, p. 169.

4. Tower, D. B.
The administration of gamma-aminobutyric acid to man: Systemic effects and anticonvulsant action. In Roberts, E. (Ed.) Inhibition in the Nervous System and Gamma-Aminobutyric Acid. Oxford, Pergamon, 1960, p. 562.

5. Tower, D. B.
Some neurochemical aspects of cortical neurobiology. In Tower, D. B. and Schadé, J. P. (Eds.) Structure and Function of the Cerebral Cortex. Amsterdam, Elsevier, 1960, p. 411.

6. McKhann, G. M. and Tower, D. B.
Oxidative metabolism of incubated cerebral cortex slices from pyridoxine-deficient kittens. Amer. J. Physiol. in press.

7. McKhann, G. M. and Tower, D. B.
The regulation of γ-aminobutyric acid metabolism in cerebral cortex mitochondria. J. Neurochem. in press.

8. McKhann, G. M. and Tower, D. B.
A modified manometric vessel for special studies on tissue metabolism. Anal. Biochem. in press.

9. Tower, D. B., Wherrett, J. R. and McKhann, G. M.
Functional implications of metabolic compartmentation in the

central nervous system: Interrelationships of cellular proteins and amino acids, fluid spaces and electrolytes, and oxidative metabolism, as delineated by ammonia. In Kety, S. S. (Ed.) Regional Chemistry of the Nervous System. Oxford, Pergamon, in press.

10. Albers, R. W., Koval, G., McKhann, G. M. and Ricks, D. Quantitative studies of in vivo γ-aminobutyrate metabolism. In Kety, S. S. (Ed.) Regional Chemistry of the Nervous System. Oxford, Pergamon, in press.

Honors and Awards: None.

Serial No. NINDB-14(c)

1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Isolation, Fractionation, Characterization and Metabolism of Brain Nucleic Acids (RNA and DNA).

Principal Investigators: Dr. M. B. Sporn and Dr. C. W. Dingman.

Other Investigators: Dr. D. B. Tower

Cooperating Units: Dr. Theodor Wanko, NINDB Section of Ophthalmological Cytology

Dr. Jan Cammermeyer, NINDB Section of Experimental Neuropathology

Man Years (calendar year 1960):
Total: 1.2
Professional: 1.0
Other: 0.2

Project Description:

Objectives: To carry out detailed studies on the characteristics and metabolic activity of cerebral DNA and RNA.

Methods: Involve the use of whole brains from young adult male rats. See under Major Findings for specific methods.

Patient Material: None.

Major Findings: This project was activated in July 1960, hence the data cover less than 5 months at the time of reporting.

(1) Attention has been devoted to developing a reproducible method for isolating the various fractions of pure, undegraded cellular particles (nuclei, mitochondria and microsomes) from whole rat brain.

(a) The brains are rapidly removed, minced and homogenized in Dounce homogenizers in a medium of 0.25 M sucrose with 0.001M $MgCl_2$ and 0.0008 M K-phosphate buffer, pH 6.7 - 6.8.

(b) Heavier tissue components (nuclei, myelin, red cells, capillary and whole tissue fragments) are sedimented by differential

centrifugation, leaving mitochondria, microsomes and cell fluid in suspension.

(c) The crude nuclear fraction is purified by suspension in 2.2 M sucrose, containing 0.001 M $MgCl_2$, 0.0035 M K_2HPO_4 and 0.0007 M Na_2ATP at pH 6.7 - 6.8, and centrifuged at 40000 x g for 2 hrs. Under these conditions only the nuclei sediment, leaving contaminating non-nuclear components in the supernatant.

(2) The nuclear fraction so obtained is of relatively low yield (about 20% of the non-endothelial nuclei of brain) but of high purity. Under phase-contrast microscopy, the preparations show many large nuclei with prominent nucleoli (? neuronal) as well as many smaller nuclei (? glial). The procedure provides a nuclear fraction contaminated with only about 0.05% of the total mitochondria (as estimated by cytochrome oxidase assay, using spectrophotometric measurement of oxidation of reduced cytochrome c at pH 7.3) and a similarly low contamination with microsomal membranes, or endoplasmic reticulum (as estimated by cholinesterase assay, using titrimetric measurement of acetylcholine hydrolysis at pH 7.4). Electron and light microscope evaluation of these preparations is in progress by Drs. Wanko and Cammermeyer.

(3) DNA and RNA determinations on whole brain and on each subcellular fraction are carried out by an adaptation of the method of Scott et al (J. Histochem. Cytochem. 4:1, 1956). The sample is precipitated at 0°C with 0.4 N perchloric acid (PCA) and washed repeatedly with 0.2N PCA. Lipids are extracted twice with absolute ethanol, twice with chloroform-methanol and twice with methanol. RNA is then extracted by hydrolysis with 1N NaOH at room temperature for 1 hr., followed by precipitation of solubilized protein and DNA with HCl. DNA is then extracted by 1.6 N PCA at 60° C for 7 min. Both RNA and DNA are then measured individually by ultraviolet absorption at 260 mμ.

(4) Total rat brain RNA was 1.36 (± 0.03) mg./g. wet weight and DNA 1.39 (± 0.06) mg./g. wet weight (means for 8 animals).

(5) Extension of these studies to the isolation of mitochondrial, microsomal and supernatant (cell fluid) fractions by differential and density gradient centrifugation is in progress. Purity will be assessed by specific biochemical characteristics outlined above plus morphological controls (electron microscopy). Isolation of microsomal RNA will then be undertaken and compared with similarly isolated liver microsome RNA for various characteristics.

Significance of Project: Neuronal RNA and RN-protein (Nissl substance) differ from cytoplasmic RN-proteins of other cells (e.g. liver) in their reactions with acridine dyes. In addition brain RNA exhibits rapid turnover, changes with state of neural activity, and sensitivity to a variety of toxic agents. Finally a number of vital processes, notably protein synthesis, depend upon cytoplasmic RNA. Hence the studies under way here are aimed at providing a more detailed approach to the understanding of these facets of brain RNA.

Proposed Course: To continue studies outlined above.

Part B included: No

Serial No. NINDB-15(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Muscle Proteins in Neuromuscular Diseases

Principal Investigator: Dr. Beni Horvath

Other Investigators: Dr. K. G. Henriksson, Mr. J. B. Proctor.

Cooperating Units: Dr. G. M. Shy, NINDB Medical Neurology Branch

Dr. I. Klatzo, NINDB Section on Clinical Neuropathology

Dr. K. Laki, NIAMD Section on Physical Biochemistry (LPB).

Man Years (calendar year 1960):
Total: 2.8
Professional: 1.4
Other: 1.4

Project Description:

Objectives: To study the comparative biochemistry of human and experimental animal muscles in health and disease, with special attention to muscle proteins which are of known or suspected structural and functional importance. To investigate the distribution, physicochemical and immunological properties of such proteins. And to characterize biopsy specimens obtained from patients in terms of the properties of muscle proteins derived from such specimens, in order to obtain a more complete description of metabolic disturbances known or suspected to occur in neuromuscular disorders.

Methods: See previous reports and under Major Findings.

Patient Material: From NINDB patients admitted for other purposes.

Major Findings:

A. Immunological Studies with Clam Tropomyosin A:

(1) Based upon the unusual immunological properties of structural

muscle proteins reported previously [cf. 59-NINDB-7(c)] and the methods developed in this laboratory for detailed study of structural muscle proteins, the time course of antibody titers in rabbits following the administration of Clam Tropomyosin A have been followed. Injections given every other day for two weeks result in titers comparable to the highest reported in the literature for other antigens, but the titers decline rapidly to low levels, with a half-life of approximately one week. Months after the titer was maximal, repeat injections of Clam Tropomyosin A causes a rise in titer. This "booster" effect and the extent of the original titer depend on the dose injected. An attempt was made to maintain a titer by injections repeated at weekly intervals. The titer achieved and the time course of the rise corresponded to the theoretical expectation from the response to a single injection and the assumption of a single exponential decline of titer with a half-life of one week. But the titer could not be maintained (as would follow from the same assumptions) but fell at about the same rate as it did after single or closely-spaced injections.

(2) Rabbits exposed to closely-spaced injections occasionally showed weight loss and death within a few months after the injections, whereas those injected at weekly or monthly intervals regularly exhibited weight loss and death. Such animals were autopsied and the muscles and internal organs examined pathologically. Glomerulonephritis was a constant finding and muscle lesions were occasionally seen, but if present were minimal.

B. Extraction and Fractional Analysis of Muscle Proteins:

(1) On the basis of the observation of E. Helander (Acta Physiol. Scand. 41 (suppl. 141), 1957) that 1.1 M potassium iodide (KI) extracts virtually all the proteins of normal rabbit muscle, a method has been developed for the analysis of muscle biopsy specimens in terms of major protein fractions. These fractions are similar to those previously described [cf. 58-NINDB-36(c)] except that the disturbingly large "alkali-soluble" protein fraction, which defied further characterization, is virtually absent, appearing instead as a small "alkali-soluble, KI - insoluble" fraction and a small "KI-soluble, KCl - insoluble" fraction. The major advantage of the new method is that having all proteins initially in solution and gradually eliminating them as precipitates under well-controlled conditions, one has confidence that no fraction is underestimated because of faulty extraction. The cumulative errors inherent in calculations by both our old method and by Helander are also eliminated by determinging protein nitrogen directly on the various fractions, rather then estimating them by difference between total nitrogen found in two

fractions. By using the same micro modification of the Nessler method employed for determination of immune precipitates [cf. 59-NINDB-7(c)], the size of biopsy samples suitable for complete analysis can now be reduced from 0.3 g. to 0.017 g. or less, or alternatively extracts from larger samples can be utilized for other studies as well.

(2) The procedure was developed on rabbit and mouse muscle and has now been applied to 40 human muscle biopsy specimens, with the data serving as a base line study for comparisons of normal human and animal muscle on the one hand and for comparisons of diseased muscle with the normal both in man and in the mouse or rabbit. The 40 human biopsy samples were obtained from patients representing 6 groups of about equal size, representing a control group and groups for each of 5 neuromuscular diseases. A preliminary statistical evaluation yielded a measure of accuracy of the procedure. It appears that averages of triplicate determinations, as routinely done, have a standard error of about 0.5 mg. N/g. or 2 to 5%, and that 95% confidence limits for the regression lines calculated for the normal points vary from 0.2 to 1.5 mg. N/g. When the numbers of samples in each group are somewhat greater, the numerous determinations for neuromuscular disease patients which fall outside these limits may be assigned statistical significance in terms of differences between the normal and disease states.

Significance of Project: This project is part of an integrated effort, involving many disciplines, directed toward the elucidation of the nature of myopathies such as muscular dystrophy, myotonia and myasthenia gravis. Since the functioning of normal muscle (contraction and work) has been shown to be dependent upon physicochemical changes of actomyosin with ATP and other proteins, the weakness and wasting associated with myopathies may be profitably investigated in terms of the structure, synthesis and functioning of this major muscle protein. Myopathies belong to the group of degenerative diseases, and hence a better understanding of diseases of skeletal muscle may permit development of approaches to such diseases of other muscles, e.g. heart, blood vessel and uterine musculature, which are less well understood in biochemical terms of component proteins than skeletal muscle.

Proposed Course: To continue both the immunochemical investigations of well-defined, purified muscle proteins and the analyses of muscle biopsy specimens from patients. To extend studies on the latter to include ultracentrifuge and electrophoretic characterization of the proteins present. And to combine the immunological and physicochemical investigations of the extracts of biopsy specimens by immuno-electrophoresis of the various fractions, which may be expected to yield greater resolution and hence detection of small differences between

normal and diseased muscle samples.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications:

1. Horvath, B., Miquel, J., Klatzo, I. and Proctor, J. B.

Immunochemical studies on muscle proteins. Neurology 10: 457 (1960).

2. Miquel, J., Horvath, B. and Klatzo, I.

A chromatographic technique for the quantitative study of the precipitin reaction. J. Immunol. 84: 545 (1960).

3. Horvath, B. and Proctor, J. B.

Muscular dystrophy: Quantitative studies on the composition of dystrophic muscle. Res. Proc. Assoc. Nerv. Ment. Dis. in press.

4. Klatzo, I., Horvath, B. and Engel, W. K.

Observations on myosin in the abnormal muscle using fluorescent antibody technique. Res. Proc. Assoc. Nerv. Ment. Dis. in press.

Honors and Awards: None

Serial No. NINDB-16(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of muscle cholinesterase and its inhibitors.

Principal Investigator: Richard L. Irwin

Other Investigators:

Cooperating Units: Laboratory of Chemistry of Natural Products, National Heart Institute

Man Years: (calendar year 1960)
Total: 1.0
Professional: .75
Other: .25

Project Description:

OBJECTIVES: During the previous course of this project the pharmacology of several new compounds, derivatives of the alkaloid galanthamine, have been studied. These substances were found to inhibit muscle, brain, and plasma cholinesterase and to increase the contraction of skeletal muscle. This group of compounds thus possess some potential as useful therapeutic drugs. During the past year many other derivatives of galanthamine have been similarly studied with the objective of finding active substances which are suitable for clinical trial and offer promise in the treatment of neurological disorders. A further and continuing objective of this project is the investigation of the relationships between cholinesterase and its inhibitors in regard to transmission processes and blockade by drugs.

METHODS EMPLOYED: Standard Warburg and potentiometric automatic titration methods have been used to determine in vitro cholinesterase activity. The latter method has increased tenfold the rate at which the data can be obtained. The muscle potentiating activity has been determined in vivo in animals in order to determine the potency, duration of activity, and undesirable physiological changes. Acute toxicities have been studied in several species. Isolation from plant sources and

preparation of the derivatives were accomplished by the Laboratory of the Chemistry of Natural Products of the National Heart Institute.

MAJOR FINDINGS: Of the numerous compounds investigated, three have been found which have properties which make them of interest from the view point of clinical usefulness in the treatment of myasthenia gravis: (1) Deoxydemethyl lycoramine methiodide (2) The dimethyl carbamate of deoxydemethyl lycoramine methiodide and (3) The dimethyl carbamate of deoxydemethyl lycoramine hydrochloride. Each of these compounds have cholinesterase inhibiting potency equal to or greater than neostigmin or physostigmine and much greater than pyridostigmin or edrophonium. Each of the three compounds have marked activity in preventing or alleviating neuromuscular block produced by non-depolarizing blocking agents.

Deoxydemethyl lycoramine methiodide differs from the carbamate compounds in having a short duration of action. Maximal muscle potentiation can be produced and disappear within 5 minutes. These properties, a brief duration of action and a cholinesterase inhibiting potency greater than edrophonium, indicate that this compound may have advantages over edrophonium as a test substance for the diagnosis of myasthenia gravis.

The dimethyl carbamate of deoxydemethyl lycoramine methiodide has the greatest potency of any of the compounds tested. Compared to neostigmin muscle potentiation is greater and the duration of action is longer. Toxicity after parenteral administration of the compound, as judged by LD_{50} data from animals, is also greater than that of neostigmin. Toxicity in rats following oral administration is less than for neostigmin, indicating poor absorption. Respiration is stimulated and blood pressure is temporarily lowered to a small extent, both respiratory and vasomotor effect are prevented by a small dose of atropine. Atropine at 0.1 mg/Kg protects against doses which are lethal in 100% of the animals.

The dimethyl carbamate of deoxydemethyl lycoramine hydrochloride has in general the same type of activity as the methiodide salt. The chief difference being that the hydrochloride has better absorption following oral administration. The potency of the hydrochloride in producing muscle potentiation is about 1/4 that of the methiodide, but greater than physostigmine. The hydrochloride has a delayed onset of action, thirty minutes being required for maximal effect. The duration of action is similar to that of neostigmin or physostigmine. The hydrochloride of the dimethyl carbamate of deoxydemethyl lycoramine has about 1/10 the toxicity after parenteral administration as does the methiodide and is about 1/4 as toxic as physostigmine after oral administration.

The dimethyl carbamate of deoxydemethyl lycoramine methiodide has been presented for clinical trial. As judged by animal experiments, the therapeutic ratio appears to be similar to that of neostigmin. Whether any of these compounds will offer advantages over the therapeutic agents now in use is as yet unknown and will depend upon a continued clinical trial and future subacute toxicity studies.

Absorption following oral administration without passage across the blood brain barrier into the brain would be a desirable quality in a drug which is used for treatment of myasthenia gravis. A study is in progress to determine to what extent the galanthamine derivatives and related drugs pass the blood brain barrier and inhibit esterases in the brain.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: This project relates to the development and testing of clinically useful drugs for the treatment of myasthenia gravis.

PROPOSED COURSE OF PROJECT: To continue to search for active compounds which may be useful in the treatment of myasthenia gravis.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Irwin, R.L., and Smith, H.J.: The activity of galanthamine and related compounds on muscle, Arch. int. pharmacodyn. 127:314, #3-4, September, 1960.

Irwin, R.L., and Smith, H.J.: Cholinesterase Inhibition by Galanthamine and Lycoramine, Biochemical Pharmacology, 3:147, August, 1960.

Serial No. NINDB-17(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Interaction between macromolecules and pharmacologically active chemicals.

Principal Investigator: Richard L. Irwin

Other Investigators: Eberhardt G. Trams
Robert A. Resnick

Cooperating Units: Section of Lipid Chemistry, NINDB
Section of Opthamology Chemistry NINDB

Man Years (calendar year 1960):
Total: 1.0
Professional: .75
Other: .25

Project Description:

OBJECTIVES: The majority of chemicals which alter biological functions do so by interaction with cellular components. These components are commonly referred to as receptor sites, and have been shown to have a high degree of specificity. Almost nothing is known of the chemical composition of the sites, either as to which cellular macromolecules are involved or what molecular or submolecular force is responsible for the interaction. The objective of this project is to determine which macromolecular components found in cells react with physiologically active chemicals.

METHODS EMPLOYED: Pharmacologically active chemicals often produce their effects at concentrations that are too low to be detected by chemical methods. Bioassay procedures are thus used to determine whether an active substance has reacted with a macromolecule. This type of method necessitates control with other macromolecules which do not react with the active drug. Suitable experimental designs have not been reported and thus the methodology of using bioassays for this purpose is being developed.

MAJOR FINDINGS: This project is a new one and no definitive results are available. Preliminary results indicate that certain glycolipids sequester some di-quaternary compounds which have marked pharmacological effects. The biological activity of mono-quaternary compounds does not appear to be changed by the presence of the glycolipids.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: A greater understanding of how drugs act with cellular molecules may: (1) Make predictable the design of the new therapeutic agents in the treatment of neurological disorders. (2) Lead to a greater understanding of cell function in neurological diseases.

PROPOSED COURSE OF THE PROJECT: To continue to pursue the objectives previously stated. The immediate course is the development and standardization of the quantitative bioassay methods.

Part B included No

Serial No. NINDB-18(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Neurohumoral factors in Cystic Fibrosis of the Pancreas

Principal Investigator: Edward L. Eyerman
Richard L. Irwin
Other Investigators:

Cooperating Units: Pediatric Metabolism Branch-NIAMD
Cystic Fibrosis Study-NIAID

Man Years (calendar year 1960):
Total: 1.0
Professional: .75
Other: .25

Project Description:

OBJECTIVES: Many investigators postulate that excessive parasympathetic stimulation of exocrine glands is the mechanism responsible for the high electrolyte and viscid polysaccharide content of secretions in cystic fibrosis of the pancreas. Direct evidence in support of this postulate is lacking. Roberts (Lancet, November 28, 1959) has hypothesized that excessive exocrine gland activity results from a defective enzymatic breakdown of the stimulating substance released by nerve endings which innervate the gland. A stimulating neurohumor not completely inactivated at its site of release might be found in the secretion of the gland it stimulates. Thus we have assayed sweat, a secretion peculiarly abnormal in the disease, for biological activity to determine what is present that might be characteristic of cystic fibrosis of the pancreas.

METHODS EMPLOYED: Patient subjects with and without cystic fibrosis of the pancreas (supplied by Pediatric Metabolism Branch NIAMD and by Cystic Fibrosis Study of NIAID) are sweated thermally in plastic bags by the method of DiSant-Agnese for a timed period. Sweat volume, PH, sodium and potassium are measured. Samples are acidified and assayed immediately or frozen to preserve activity. The activity of the sweat is determined by bioassay using the clam-heart of

venus mercenaria and, where feasible, the eserinized frog rectus muscle.

MAJOR FINDINGS: Cholinergic activity due to a choline ester, presumably acetylcholine, of a rather surprising amount, has been found in sweat samples from all but one of twelve patients with cystic fibrosis of the pancreas, in contrast to lower activity obtained from non-cystic samples. That a choline-ester rather than electrolyte content or choline itself causes the observed activity of the samples is clear from alkali-denaturation and other tests.

The average activity for the twelve cystic patients, expressed as acetylcholine, is 0.075 micrograms/ml of sweat. A similar value for twelve patients without cystic fibrosis of the pancreas is 0.0054 micrograms/ml. Three cystic patients gave very high values, averaging 0.22 micrograms/ml. The highest value obtained for a non-cystic patient was 0.015 micrograms/ml. The data is rather widely scattered in the disease group and thus a greater number of experiments are needed to evaluate the significance of the data.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: This project is an attempt to determine whether cystic fibrosis of the pancreas, a disease of unknown etiology, involves neurohumoral imbalance and thus is of neurological importance.

PROPOSED COURSE OF PROJECT: To determine the exact chemical nature of the substance responsible for the demonstrated cholinergic activity of sweat. This may be possible by the use of concentration technique and chromatography as well as bioassay in other systems. Standardization in collection of sweat and further sampling from cystic subjects and non-cystic subjects, especially from adults, will be necessary to give validity to the quantitative findings.

Part B included No

Serial No. NINDB-19(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The localization of muscle fibers in a single motor unit.

Principal Investigator: Forbes H. Norris, Jr.

Other Investigators: Richard L. Irwin

Cooperating Units: None

Man Years: (calendar year 1960)
Total:
Professional:
Other:

Project Description:

OBJECTIVES: The interpretation of muscle action potentials obtained by electromyographic recording is handicapped by a lack of knowledge as to the exact anatomical distribution of fibers of a single motor unit. The objective of this project was to develop suitable methods and to study the anatomical distribution of the fibers of a single motor unit and its electrical activity.

METHODS EMPLOYED: Single axon preparations were made in ventral roots of the rat spinal cord. During electrical stimulation of this axon, the innervated muscle was explored by means of glass micropipette electrodes. The muscle fibers supplied by the axon were located through evoked, intracellular, depolarization potentials. Thus, a map could be made of the muscle in cross-section showing the exact location of the muscle fibers in a motor unit. It was possible to prepare 21 single axons, and to record intracellularly from 2 or more muscle fibers supplied by the axon in 10 experiments. The single axons were not salvaged for histological proof that only a single axon was being stimulated in each instance. In most experiments, several axons were probably stimulated but only one innervated the muscle being explored; this was determined by demonstrating an all-or-none tension response over a wide range of stimulus intensities. In the 10 experiments cited above, muscle fibers of the same motor

unit were found throughout the muscle in most instances.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: This study indicates that singly or in small groups the muscle fibers of a motor unit are widely dispersed in the muscle. The method could have selected axons of large diameter, supplying larger numbers of muscle fibers than smaller axons with smaller motor units. There is some evidence that motor unit size is related to function. Thus, changes of motor unit "territory" in neurological diseases could reflect some specific functional impact of these diseases as well as changes in actual motor unit size.

PROPOSED COURSE OF PROJECT: Above results are being prepared for publication in a scientific journal.

Part B included: No

ADDENDUM TO: Serial No. NINDB-3 (c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: A study of neuromuscular transmission in reference to myasthenia gravis.

Principal Investigator: Richard L. Irwin
Peter T. Rowley

Other Investigators: Jay B. Wells

Cooperating Units: NINDB-88C-1956

Man Years:
Total: .66
Professional: .33
Other: .33

Part B

Honors, Awards, and Publications

Rowley, P.T., Wells, J.B., and Irwin, R.L.: Tension response of mammalian muscle to intra-arterial acetylcholine, The American Journal of Physiology, 198:507, #3, March, 1960.

ADDENDUM to: Serial No. NINDB-21 (c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The action of neuromuscular blocking drugs on directly stimulated innervated and denervated muscle.

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells

Cooperating Units: None

Man Years: (calendar year 1958)
Total: .66
Professional: .33
Other: .33

Patient Days: (calendar year 1958)

Part B

Honors, Awards, and Publications

Wells, J.B., and Irwin, R.L.: The effect of succinylcholine on isotonic contraction of directly stimulated mammalian skeletal muscle, Accepted for Publication in the Journal of Physiology on October 24, 1960.

ADDENDUM TO:

Serial No. NINDB-22 (c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Maryland
4. Continuation of NINDB-20(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Blood and tissue cholinesterases in neuromuscular blockade.

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells and Henry J. Smith

Cooperating Units: None

Man Years: (calendar year 1959)
Total: 0
Professional: 0
Other: 0

Patient Days: 0 (calendar year 1959)

Part B.

Honors, Awards, and Publications

Publications other than abstracts from this project:

"The Effects of Selective Inhibition of Muscle and Plasma Cholinesterase on Neuromuscular Block." Proceeding of the Second (1959) International Symposium on Myasthenia Gravis. Arthur H. Thomas Publishing Company (In Press), Richard L. Irwin, Jay B. Wells, and Henry J. Smith, III.

Addendum to NINDB-31(c)-1959
1. Medical Neurology Branch
2. Section of Neurological Disorders Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Thyroid Function and Neuromuscular Disease.

Principal Investigator: Andrew G. Engel

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

This project was completely described in the previous Annual Report and has now reached completion. Dr. Engel has now left the Institute and no further studies are anticipated in this regard.

Proposed Course of Project:

None.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Engel, A.: Thyroid Function & Periodic Paralysis. American Journal of Medicine. (In Press)

Engel, A.: Thyroid Function and Myasthenia Gravis. JAMA Archives of Neurology and Psychiatry. (In Press)

Honors and Awards relating to this project:

None.

Serial No. NINDB-20(c)-1960
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Brain Scanning with Radio-iodinated Antifibrinogen (RIAF-Encephalography)

Principal Investigators: Giovanni Di Chiro, M.D.

Other Investigators: Drs. William Bale and Irving Spar of the Rochester University School of Medicine and Dentistry and Mr. William Matthews.

Cooperating Units: The University of Rochester Atomic Energy Project, New York

Man Years: (calendar year 1960)
Total: .40
Professional: .20
Other: .20

Project Description:

Objectives: It is logical to assume that a group of endocranial lesions in which there is abundance of fibrinogen or fibrin (fresh or organized intra- and extra-cerebral bleedings, thromboses, infarcts) should present a specific uptake of radio-labelled antibodies to fibrinogen. In addition from previous experiences gained from transplantable rat tumors and spontaneous dog tumors there is some evidence that tumors of sarcomatous type show a "significantly" specific uptake of radio-labelled antibodies to fibrinogen. The radio-labelled antifibrinogen should therefore be, at least theoretically, useful for diagnostic radioactive scanning studies in cases of endocranial lesions with "paramount fibrinogen involvement" and in cases of cranial tumors of sarcomatous type.

Methods Employed: Antibodies to human fibrinogen prepared from immunized rabbits and made radioactive by "in vitro" coupling with I^{131} are injected intravenously in patients suspected of having brain lesions. After a congruous period of time these patients are "brain scanned". Emphasis is put on a comparison with the results obtained, possibly in the same patients, with RISA-brain scanning. The amount

of activity injected, the scanning technique and other details are the same as with the technique of RISA-encephalography. In addition patients with thrombophlebitis of the limbs are injected with radio-iodinated antifibrinogen (RIAF) to study the specific uptake in cases of "obvious" lesions in which the fibrinogen is involved.

Patient Material: Up to now twelve patients have been used for the present study.

Major Findings: Twelve patients have been injected up to now with RIAF. No untoward affects have been observed. Five of these patients had verified brain tumors. These five cases also received RISA-encephalography. Three glioblastomas were detected and localized with RISA more accurately than with RIAF. One case of astrocytoma was very nicely localized by means of RIAF, probably with better "outlining and definition" than with RISA. In this specific case one has the impression looking at the scan that a highly selective uptake of the labelled globulin has taken place. In a fifth case a patient with multiple intracranial fibrosarcomas, one of the lesions was detected and localized with RIAF while it had been missed about ten weeks before with RISA. We have reason to believe that at the time of the RISA scanning the lesion which was detected afterwards with RIAF was already present. This lesion can be in fact "retrospectively" recognized in the RISA scan.

Extremely interesting results have been obtained with two patients with thrombophlebitis of the thigh.

A very selective uptake of the antibody to fibrinogen was found in a specimen of a venous thrombus of a patient who had received the RIAF. The activity in the thrombus-specimen was much higher than the activity in the rest of the blood.

Significance to Neurological Research: This method should represent a new diagnostic tool especially helpful in that large and often "elusive" group of conditions loosely referred as "cerebral vascular accidents".

Proposed Course of Project: Added experience is necessary.

Part B included Yes ☐ No ☒

Addendum to NINDB-24(c)-1959
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: RISA (radio-iodinated serum albumin) Encephalography and Conventional Neuroradiological Methods

Principal Investigators: Giovanni Di Chiro, M.D.

Other Investigators: None

Man Years:
Total: .40
Professional: .20
Other: .20

Project Description:

Major Findings:

1. RISA-encephalography is a valuable diagnostic method for detection and localization of cranial lesions.

2. Some tumoral lesions, most frequently glioblastomas, missed with conventional neuroradiological studies (plain x-rays and laminagraphy of the skull, carotid and vertebral angiographies, air studies) are detected with RISA-encephalography.

3. Conversely, some intracranial lesions, of different pathological nature, detected and localized with conventional neuroradiology are missed by RISA.

4. Conventional neuroradiology remains unmatched for assessing the nature of the different lesions.

5. RISA-encephalography is sometimes more accurate than other neuroradiological studies for determining the extension of certain lesions, mostly "gliomatous" but sometimes also of a different nature as meningiomas.

6. Occasionally the presence of multiple lesions (multiple metastases, multiple meningiomas) is established with RISA-encephalography and not with conventional neuroradiological methods.

7. RISA-encephalography carries, at least at present, a certain degree of "unpredictability". In fact certain lesions, occasionally large ones, are sometimes unexplainably missed. In addition the possibility of "false positive" findings in RISA-encephalography is real; an increased radioactive uptake due to

"nonexpanding" or "progressive" lesions may mislead the interpreter.

8. RISA-encephalography should be used "together" with other neuroradiological methods and not "in lieu" of them.

Proposed Course of Project: The comparative study is at present continuing on the basis of new cases and added experience.

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, Giovanni: RISA (radio-iodinated serum albumin) Encephalography and Conventional Neuroradiological Methods. (Accepted for publication and to be published in the near future as a Supplement of Acta Radiologica, Stockholm, Sweden.)

Honors and awards relating to this project: None

Addendum to NINDB-26(c)-1959
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Atlas of Fine Encephalographic Anatomy

Principal Investigators: Giovanni Di Chiro, M.D.

Other Investigators: None

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, Giovanni: An Atlas of Detailed Normal Pneumoencephalographic Anatomy. (Accepted for publication and to be published soon by Charles C. Thomas, Publisher, Springfield, Illinois)

Honors and awards relating to this project: None

Addendum to: NINDB-27(c)-1959
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Sella and the Pituitary in the Coronal Plane

Principal Investigators: Giovanni Di Chiro, M.D. and Joseph Morel, R.T.

Other Investigators: None

Man Years:
Total: .40
Professional: .20
Other: .20

Project Description:

Major Findings:

1. In over 90 per cent of the cases it is possible to reproduce roentgenographically, the width (third dimension) of the sella turcica in conventional X-ray studies of the skull. With help of laminagraphy, the width of the sella turcica may be established roentgenographically in 100 per cent of the cases.

2. The three dimensional study of the sella turcica has many advantages as shown in specific cases which are reported. Actually an assessment of the true anatomical conditions of the sellar region can be obtained only three-dimensionally.

3. Until now, the size of the sella has been determined by X-ray either with "linear" or with "area" measurements. Now the size of the sella may be measured "volumetrically". A simple and fast method of measuring the volume of the sella by X-ray has been suggested. The method has proven to have a very satisfactory accuracy when tested with anatomical verifications.

4. It has been proved that the width of the sella as determined by X-rays corresponds to the width of the pituitary gland. As a matter of fact, the width of the pituitary corresponds more closely to the X-ray width of the sella than the length and depth of the pituitary correspond to the X-ray length and depth of the sella.

5. A roentgenographic method of measuring the volume of the pituitary gland "in vivo" is now feasible.

Proposed Course of Project: At present, further studies are being

carried out on the radiographic measurement of the sellar volume. The present objective of anatomical and radiographic studies is mainly to establish if a relationship exists between the size of the sella (and indirectly, the size of the pituitary) and age, sex, size of the skull, thickness of the calvaria, and size of the paranasal sinuses.

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, Giovanni: The Width (Third Dimension) of the Sella Turcica, The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine 84, 26-37, 1960.

Honors and awards relating to this project: None

Addendum to NINDB-28(c)-1959
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Bilateral Angiographic Evaluation of the Superficial Veins and Sinuses of the Brain

Principal Investigators: Giovanni Di Chiro, M.D.

Other Investigators: None

Man Years:
Total: .40
Professional: .20
Other: .20

Project Description:

Additional Findings:

1. The angiographic pattern of the convexity veins is not arranged as "at random" as it is generally believed.

2. The pattern of superficial veins discharge in the two hemispheres is only seldom comparable in one and the same patient. In the great majority of the cases, this pattern is different in the two hemispheres, often markedly so.

3. In many cases, one of the three main superficial venous channels (vein of Trolard, Sylvian vein and vein of Labbé) is predominant in one hemisphere while in the opposite hemisphere another of these three channels predominates.

4. The occurrence of a right predominant Trolard and of a left predominant Labbé is "significantly" frequent (over 60 per cent of the cases).

5. Attempts have been made to relate the angiographic findings of hemispheric superficial venous patterns with the handedness, the Wada test, the cortical pharadic stimulation and the postoperative speech disturbances in order to try to establish whether or not a relationship exists between the pattern of the superficial venous discharge and the hemispheric dominance. At present the findings are being interpreted with this purpose in mind.

6. It has been angiographically established that the blood of the superior longitudinal sinus and of the sinus rectus enters in

over 70 per cent of the cases into the right and left transverse sinuses dividing roughly in equal amounts between these two sinuses.

7. In some cases, one of the transverse sinuses predominates and it receives most of the blood from both hemispheres.

8. In few cases there is a complete lateralization of the discharge of the two transverse sinuses; that is the right transverse sinus receives blood only from the right hemisphere and the left transverse sinus receives blood only from the left hemisphere.

9. In exceptional cases it is possible to demonstrate angiographically that the sinus rectus discharges its blood into the left transverse sinus and the superior sagittal sinus into the right transverse sinus. We have therefore an angiographic confirmation that in some cases the left jugular vein contains mostly blood from the deep parts of the brain while the right transverse sinus contains mostly blood from the surface of the brain.

Proposed Course of Project: Additional angiographic experience. Anatomical studies.

Addendum to: NINDB-30(c)-1959
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Radiographic Study of the Soft Tissues in the Different Muscle Diseases

Principal Investigators: Giovanni Di Chiro, M.D.

Other Investigators: Mr. Fred H. Meiller

Man Years:
Total: .40
Professional: .20
Other: .20

Project Description:

Major Findings: Work on this "long range" project has been continued.

At present after numerous "tries" with different techniques for the roentgenographic studies of the soft tissues of the limbs the following technique has been chosen and standardized. Kv and mas values in the comparatively high range are used. The light emission of the intensifying screen located in the back of the X-ray cassette is blocked with a black piece of paper placed between the screen and the X-ray film. The X-ray films obtained with this technique are afterwards reproduced, to enhance the contrast and detail, with a Logetronic equipment.

A few normal controls have been X-rayed specifically for this project and, to avoid unnecessary X-ray exposure of a high number of normal controls, X-ray films of limbs taken for different purposes of patients not affected by neuromuscular diseases have also been used. In this way a fairly good idea of the roentgenographic appearance of the soft tissues of limbs in individuals not affected by neuromuscular disease in different age groups and of different size has been obtained.

The soft tissues of the limbs of over one hundred fifty cases of different neuromuscular diseases have been studied readiographically and the different findings are now being analyzed.

Proposed Course of Project: Establish whether or not specific roentgenographically demonstrable changes of the soft tissues of the limbs in patients with different neuromuscular diseases exist.

ANNUAL REPORT
OPHTHALMOLOGY BRANCH
NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND BLINDNESS

Calendar Year 1960

The past year brought considerable change in the ranks of investigators of this Branch. Most acutely felt, and distressing, has been the resignation of Dr. M. G. F. Fuortes from the position as Chief of the Neurophysiology Section. Dr. Fuortes accepted an appointment as Director of the "Instituto Neurologico" in Milano, Italy, and returned to his home country in September. Dr. Fuortes held a key position in the Ophthalmology Branch. His share in its scientific efforts carried great weight indeed, and his contributions were probably the most outstanding and best known of those produced in the Branch in the past five years. The quality of his work, his clear mind and reach in understanding problems only faintly related to his specific line of research, stimulated many competent electrophysiologists to visit him, discuss their work and seek his advice. Upon the suggestion of Dr. Fuortes, the eminent Dr. W.H. Rushton was appointed as a Visiting Scientist in the fall of 1959. Unfortunately, after a most fruitful year of investigative work in the Institute and lecturing throughout the country, Dr. Rushton had to terminate his stay in Bethesda recently to return to Trinity College in Cambridge, England.

There is, I am inclined to believe, ample scope for hope that these losses will be made up in part by two new appointments to the permanent staff of the Branch. Dr. S. L. Bonting, whose reputation as a biochemist in the field of cell biology is well established, was made Chief of the new Section of Cell Biology, and Dr. Peter Gouras, who worked for a year with Dr. Fuortes and for another year with Dr. Rushton in England, joined the Branch again in September 1960. Replacements of young promising ophthalmologists as clinical associates followed the usual two years renewal scheme. These ophthalmologists bring with them some new ideas and concepts of their school and take along information which might be helpful in their future. With all this change it is encouraging that almost all of the clinical associates in the last five years decided upon, or actually started, an academic career.

In this year, I feel again that emphasis on scientific accomplishment should be placed on contributions which deal with basic studies of visual processes and electrophysiological events connected with vision. Dr. Rushton completed three studies of direct measurements of visual pigments in relation to scotopic thresholds using his retinal densitometer. The technique permits the direct determination of rise and fall of visual pigments in the living human eye and a comparison between the rates of bleaching and synthesis of photosensitive pigments in the course of light and dark adaptation. Dr. Rushton concluded that there is a fixed relation between these thresholds and the fraction of opsin uncombined with retinine. The mathematically defined relation indicated that the logarithm of threshold directly depends

on rhodopsin concentration. The rod-cone kink in the dark adaptation curve always coincides with the time at which 90% rhodopsin concentration is reached.

In extensive studies on a rod monochromat, normal spectral sensitivity in cone vision was shown to be present in a small zone adjacent to the fovea, but the very high threshold, absence of color discrimination and low visual acuity (20/80) indicated abnormal cone organization. The subject had a normal rod function, and rhodopsin regeneration proceeded at a normal rate. No pigments were detected in the subjects fovea. This monochromat's dark adaptation curve could be plotted in the cone branch without contamination by cone function. Since it did not differ from the curve of a normal subject no inhibitory effect of cones on rods was evident. These findings suggest that the two visual receptors function independently.

The results of Dr. Fuortes' new studies of the activity of cells in the eye of the limulus, obtained by intracellular recording with glass micropipettes are preliminary in nature and at present available only in form of a summary. Dr. Fuortes examined the changes evoked by light in various stages of adaptation. He recorded depolarizing transients from cells of the dark adapted limulus eye following the exposure to weak light stimuli. The observations imply that one quantum may be sufficient for generation of one transient. Examination of the responses to brief flashes of light and brief depolarizing pulses before and after application of a strong light showed quick recovery of responses to the depolarizing current, but slow recovery of responses to light flashes. Background illumination increased the responses to current and decreased those to light. These and other results appear to be of great importance on first sight, but a comprehensive report cannot be given prior to a detailed evaluation of the findings by Dr. Fuortes.

The experimental part of studies conducted by Dr. K. Tansley, R. M. Copenhaver, and R. D. Gunkel was completed in 1959, but the compilation of data, the calculations based on spectral sensitivity curves and the histological examinations were carried out and reported in 1960. Three excellent studies have been submitted for publication which deal with various aspects of electroretinographic responses in pure cone retinae. Four species of ground squirrels and two species of tree squirrels were studied, all of which showed the characteristics of cone retinae and thus were well suited to study an uncontaminated photopic mechanism. The spectral sensitivity curves were double humped. Adaptation of the eye to blue light depressed the hump at 490 mu and exposure to green or orange depressed the humps at 535 mu. These results suggest the existence of two photosensitive mechanisms of different sensitivities and some sort of rivalry between the two mechanisms.

Other experiments dealt mainly with the off-effect of the squirrels' electroretinogram. In contrast to other laboratory animals, a marked off-effect is characteristic for the pure cone retinae of these species.

A decline of the off-effect at increasingly high intensities was best observed in aphakic dark adapted animals. When a high intensity stimulus was followed by a similar second stimulus after 20 seconds, the off-effect was cut down and a second b- and a-wave was abolished. Lower intensity stimuli again cut short the off-effect, but the amplitude of the second a-wave was enhanced and a b-wave could be clearly recognized. Such phenomena in the electroretinogram were described in the past by Granit and Müller-Limroth. Dr. Tansley's results strongly support Granit's superposition as to the nature of the off-effect in post excitatory inhibition. There seems to be a reactivation of P-II and possibly P-III at off and not only a disappearance of P-III leaving the remaining P-II.

When the American Red Squirrel, a tree squirrel, was studied electroretinographically and with flicker during dark adaptation, a decrease of the off-effect became obvious, in contrast to the findings in the ground squirrel. The flicker fusion frequencies of the red squirrel was considerably lower than that in the ground squirrel. A striking histological difference between the retinae of the two species (fewer ganglion cells and a less complex inner nuclear layer in the red squirrel) suggests that the higher flicker fusion of the ground squirrel is connected with a more efficient production of inhibition associated with a marked development with the inner nuclear layer in this species. From all these studies, it is obvious that much was learned about the electroretinogram by investigating the mammalian pure cone retina.

Dr. Peter Gouras joined the Branch only three months ago. With admirable efficiency and competence, he was able in this short time to proceed in important work by associating with Dr. John Armington of the Psychology Department of the Walter Reed Hospital. In their study, entitled "Adaptation of Electrical Enhancement Techniques to Clinical Electroretinography", an electronic computer technique was used to greatly improve the signal to noise ratio. The conventional ERG examination does not detect responses of less than 25 μV. Employing an averaging system for the ERG responses promises to change radically the resolution potentialities of this method. In ten patients with tapeto retinal degeneration and extinguished ERG's, responses were obtained by electronic summation. The size of the remaining visual field in these patients showed close correlation to the light energy necessary for producing a constant amplitude ERG. Spectral sensitivity curves determined on these subjects revealed predominantly photopic curves in patients with macular function and more scotopic ones in those with some rod vision. The results have theoretical implications as well as practical ones, as they do not support the theory that short circuiting through Bruch's membrane can explain the absence of electric responses in patients who ophthalmoscopically have only moderate fundus pathology.

The combination of ERG studies and biochemical investigations on rhodopsin carried out in Dr. S. L. Bonting's section holds great promise. Dr. Gouras observed that the ERG of albino rats appeared at 12 days, rapidly increased to the 21st day and subsequently increased at a slower rate. a- and b-waves developed at different times. Appearance of the electrical response is closely related to the increasing rhodopsin

concentration. In recent studies, a technique was worked out to determine the dark adaptation curve for the rat, using ERG criteria. The dependency of the dark adaptation curve on bleaching procedures parallels the observations as seen in psycho-physical curves in man. Since such studies can be combined with biochemical examinations, it is obvious that important information can be expected from this experimental approach.

In another study, Dr. Gouras plans to investigate the coding of color and intensity by the vertebrate retina at the level of the ganglion cells after data have been collected on the dependency of ganglion cell discharge on the rate of changes of photic stimuli. The actual work on this project has not yet begun.

In the research activities of Dr. Rushton, Dr. Tansley and now of Dr. Gouras, Dr. Ralph Gunkel participated at an almost full time basis. There is no doubt that the accomplishments of these investigators were due, in no small part, to the unusual talent of Dr. Gunkel to construct, design or modify such devices as required in the respective studies. It cannot be stressed enough that his unique gift and his cooperative spirit has often prepared the way for a rapid progress in a given project. Dr. Gunkel gave up time needed for his own investigations in order to assist and advise the investigators and to provide the experimental conditions they desired. It is realized that he had to discontinue his own research problems dealing with retinal profile studies and the design of an objective visual acuity test.

In the area of visual processes, the biochemical approach taken by Dr. Sjoerd L. Bonting in the study of the rhodopsin cycle in the developing mammalian eye encompasses investigations of the relations of biochemical, morphological and functional changes in the developing visual receptor cell. It is an amazing achievement of Dr. Bonting that he was able to organize his section within a few months, to integrate the various complicated methods and to produce important results so soon. He found that rhodopsin begins to appear at the seventh day, to increase in concentration up to the 21st day, and then to remain constant. These changes are parallele by an increase of the length of the outer segments of rods. Dr. Gouras, working with Dr. Bonting, determined that electroretinographic responses can be detected on the tenth day and increase in amplitude up to 21 days after which they remain constant. It is interesting to learn that C3H mice, a strain which becomes blind from a hereditary degeneration of the rods starting 6-17 days after birth, show a normal development of rhodopsin until the age of 10 days; then the concentration of the pigment rapidly decreases.

Also in Dr. Bonting's laboratory, studies on various enzymes in the retina are in progress, particularly on the enzyme retinene reductase. In view of the non-specificity of the Tetrazolium technique for identification of this enzyme, experiments are under way to work out a new ultramicro-assay procedure. Dr. Bonting and Dr. Simon have begun studies on the distribution of a new enzyme, an ATP-ase, in mammalian tissues, particularly tissues of the eye and brain, to investigate its possible relationship to formation of aqueous humor and spinal fluid. In the eye,

ATP-ase activity was demonstrated in the optic nerve, choroid, and ciliary body. Most suprising to the investigators was the observation of a full inhibition of ciliary body--ATP-ase by Diamox in a concentration of 10^{-4} M. Since Diamox depresses aqueous humor formation, it can be conjectured that this ATP-ase is involved in the secretion of the aqueous humor.

Dr. Resnik continued his studies on physico-chemical characteristics of the lens proteins in cooperation with Dr. Papaconstantinou. Eight fractions of soluble proteins from the lens cortex were obtained by column chromatography and each of these fractions contained two to three components. Some of these proteins are capable of undergoing various transformations. which are pH dependant. The fractions F and G of alpha-crystalline are transformed into particles of lower molecular weight. The molecular weights of native and fully transformed alpha G crystalline determined by the approach to the equilibrium method in the ultracentrifuge are 1,086.000 and 514,000 respectively. When sedimentation and diffusion coefficients of these proteins were determined, a molecular weight of 940,000 was calculated for the native protein and 509,000 for the fully transformed alpha G crystalline. The pH induced decrease of the molecular weight of the alpha crystallin is irreversib The net charge does not seem to be altered in the transformation since boundar electrophoresis did not reveal two forms of alpha G crystallin when known mixtures were used.

A second study in Dr. Resnik's laboratory dealing with the structure of ganglioside is partly in the planning stage. Dr. E.G. Trams and Dr. R. Irwin, of the Section on Lipid Chemistry and Chemical Pharmacology of NINDB, are co-workers on this project. The molecular weight of brain ganglioside in water was around 300,000.In N, N-dimethylformamide, it fell to 3,000. Micelle formation, above a critical concentration, might explain this phenomenon which is possibly related to the solubilities of hydrophobic and hydrophilic groups in the molecule.

Reading the splendid and compendious study of Dr. van Alphen, which he modestly calls "Basic factors in refraction anomalies", is a rewarding experience. In the first part of the paper, he proves by mathematical treatment of data provided in the literature on correlations of optical elements that Steiger's theory of the origin of refraction anomalies is unsatisfactory and in no way provides an answer to the question which mechanisms are operative to bring about emmetropia and ametropia. The links between the optical elements can be explained under the assumption that the choroid behaves as a sheath muscle and is able to counteract part of the intraocular pressure. That such a mechanism exists is shown in the second part of this study in which an array of brilliant experimental procedures are listed for measuring tension and tension changes of the choroid, their effectiveness on the elasticity of the sclera and their responses to pharmacological agents. Parasympathetic stimulation results in the contraction of the ciliary body-choroid unit. In experiments on isolated strips of this tissue, acetylcholine also brings about a contraction, the extent of which depends on the tension of the preparation. Adrenergic compounds produce a relaxation. The results of these experiments provide experimental data to strengthen the statistical analysis of correlation of

optical elements in the human eye. Moreover, they suggest that investigations should be undertaken to study the relation between the "muscle sheath" and the pathology of the intraocular pressure.

Dr. Macri has made great progress in his studies on the pressure in various intraocular and extraocular vascular beds by feeding very fine polyethylene tubes into vessels such as the anterior ciliary vein, the vortex and choroidal veins and the long ciliary artery and iris artery. Sensitive transducers were used to measure the pressure in these vessels. The data were correlated with the simultaneously obtained tracings of the intraocular pressure and the general blood pressure. Experiments were carried out on the intact anesthetized cat, on enucleated perfused eyes and on isolated portions of the anterior uvea. It is the first time that pressure measurements could be carred out in the small intraocular vessels with such a refined technique. The reported results will help to clarify the obscure relation between changes in vascular pressure, aqueous humor dynamics and IOP regulations. Dr. Macri observed that pressures in the litigated anterior ciliary vein and also in apparently patent anterior ciliary veins were identical with the IOP. When the intraocular pressure was plotted as a function of the venous pressure, a linear regression equation was demonstrated. From the results obtained in experiments on intact eyes and enucleated arterially perfused eyes under various experimental conditions, it was inferred that the venous pressure is a dominant factor in the maintenance of the intraocular pressure.

In a second study, Dr. Macri sought information on the often discussed lack of correlation between the absolute levels of intraocular and general blood pressure. Changes of the blood pressure are clearly reflected in pressure changes of the ophthalmic artery, but the pressure in the circulous arteriosus iridis does not simulate the changes of the general blood pressure. The cause of this apparent dissociation is not known, nor has it been clearly demonstrated as yet that this dissociation is real and not artificial. No claims have been made as to the applicability of the many observations in the cat to the human eye, where the vascular supply differs considerably from that of the experimental animal. Therefore, the intriguing correlations between intravascular and intraocular pressures observed by Dr. Macri in the cat, will be restudied in monkeys.

This elegant technique of recording pressure in small vessels of the perfused eye was modified to study vascular responses in the isolated iris. The pressure determinations were combined with measurements of the rate of flow. A new bio-assay method was developed for examining the pharmacodynamics of autonomic mediators (epinephrine, acetylcholine) and of Diamox. According to Dr. Macri's interpretation, the arteries emerging from the circulus arteriosus of the iris in the direction of the ciliary body constricted with low doses of epinephrine and lowered the pressure in the iris ring vessel. High doses of the compound resulted in constriction of this artery. Acetylcholine decreased the pressure in the iris artery as well as the flow. Diamox in small concentrations seemed to lower the flow rate and to produce a rise of the pressure in the iris ring vessel. The inter-relationships of these responses is not clear, nor is it known whether the effects are due to specific carbonic anhydrase inhibition or to non-specific variables.

Under Dr. Macri's guidance, Dr. Luigi Scullica, guest worker from the Eye Department of the University in Rome, conducted studies on the flow pattern in the ciliary body of the albino rabbit. In previous studies, Dr. Scullica had acquired unusual skill in experimenting with the intraocular vascular system of the rabbit. In the present study, he observed that the perfusate enters the artery of the ciliary process at its anterior tip and moved from here into a large collecting vessel on the outer surface of the process. This vessel, considered to be a vein, connects with choroidal veins and a capillary bed located between the choroid and the irido-ciliary vessels. Dr. Scullica will extend his investigation to study pharmacological effects on the vascular bed of ciliary processes.

The efforts of the Cytology Section were directed mainly toward studies of the corneal endothelium of the rabbit. This mono-layer of cells plays an important role in corneal surgery. It is avascular and in direct contact with the aqueous humor. Employing a new technique, it can be isolated as a whole flat mount, ideally suitable for cytological studies. In the past, no mitosis has been observed in this tissue and the way in which growth takes place was obscure. In the recent work, it was demonstrated that the cells divided karyokinetically, mostly in the periphery of the endothelium of the growing rabbit. By estimating the cell populations and counting the dividing cells in different age groups, it was shown that cell division, cell growth and spreading of cells are the mechanisms by which this cell layer provides a continuous cover for the posterior surface of the growing cornea. Similar to the corneal epithelium and skin, mitosis in the endothelium exhibits diurnal variations, with the highest values obtained at the early morning. In view of the fact that dividing cells are either very few or absent in the fully grown animal (one to three years old), the endothelium has to be classified as a non-renewal population similar to the vascular and peritoneal endothelium.

The direct exposure of this cell layer to compounds introduced or present in the aqueous humor provides most favorable conditions for examining the effects of various agents on the endothelium in chronic experiments. Techniques are being perfected to provide for the prolonged administration of such agents and use is made of the osmotic pump, designed by Dr. Samuel Rose, of the NCI. Experiments were started in which H^3 Thymidine was infused into the anterior chamber at a slow rate for many hours. In a few preparations, the incorporation of the tracer into DNA was abundant; in other preparations, minimal. The reason for this difference is not known at present. Counts of the labelled nuclei and dividing cells are expected to provide more detailed information on the renewal and regeneration in this cell population.

Dr. Hubert Huckel, working in the same laboratory, utilized the technique of whole mounts of the endothelium to investigate the possible injurious effect of alpha chymotrypsin treatment - now widely used in cataract surgery - on healing of endothelial wounds. In the completed part of this study, the examinations were limited to eight week old rabbits. In this age group the process of regeneration as indicated by the number of dividing cells around the lesion appeared slightly depressed 24 hours after injury. But after 48 hours, no difference in the process of wound repair between the chymotrypsin treated and control eyes was revealed.

It is fully realized that a drug effect on the endothelium of young, healthy animals cannot be compared with that of patients with senile cataracts. Any conclusions, then, pointing to the harmless nature of using alpha chrymotrypsin has to be guarded.

Another study of the Cytology Laboratory, conducted in cooperation with Dr. H. Curtis of the Brookhaven National Laboratory, dealt with the effect of high energy proton microbeams which were thought to simulate the ionization pattern of cosmic primaries. A 22 MEV beam from the 60-inch cyclotron of the Brookhaven Laboratory served as the radiation source. Circumscribed areas of the lens equator were irradiated with doses varying from 1,012 to 33,000 rad through apertures which measured 25, 75 and 1000 microns in diameter. Present observations include animals which had been irradiated six months ago. Definite and sometimes striking changes were obtained only when a 1 mm. aperture was employed. Much higher doses using beams of 25 or 75 microns in diameter produced only minimal lesions in a few instances. It seems to be the impact area and not the dose which determines the extent of the damage. The 25 micron beam alone can be compared with ionization pattern of cosmic primaries where the dense cluster of secondary protons surrounding the core of the alpha particle is believed to produce biological effects. It seems that damage to the lens by cosmic primaries might be less serious than has been expected.

The study of another potentially cataractogenic agent was carried out in connection with the recent observations of circumscribed subcapsular lens opacities around the posterior pole which formed in patients with rheumatoid arthritis or other collagen diseases who were treated over prolonged periods with high doses of synthetic steroids. Groups of rats were injected daily with 5, 10 and 20 micrograms/100 grams of Decadron for four months. The effectiveness of the treatment was manifested by a dose dependent depression of weight gain. Biomicroscopic and histologic examination of the experimental animals showed that they did not differ from normal controls. In view of the claims of decreased mitotic activity in experimental animals when treated with high doses of steroids, mitotic counts were obtained and in all instances were found to be within a normal range. Blood sugar levels of the experimental animals did not differ significantly from the controls. On histological examination no lens fiber pathology was discovered. Cytological and histological studies of the cataractous lenses obtained in autopsy of a patient treated with steroids for a long time suggested that the primary lesion involves the cytoplasm of the lens fibers, particularly in the posterior cortex subcapsularly and that changes in the bow nuclei area were secondary to the fiber damage.

In Dr. Wanko's laboratories, the suture region of the lens was singled out for new electron microscopic studies. This site is of physiological and pathological interest. A most complicated organization, vaguely noticed on biomicroscopic examination, was beautifully demonstrated. The complexity of the irregular cell surface with numerous interdigitations in all planes results in an immense increase of cell surface which may account for the vulnerability of this area to various noxes. Dr. Wanko points rightly to the probable relation of this amazing localized differentiation of cell surface to the maximal dimensional changes taking place during accommodation at this part of the lens.

Material from human cataract surgery becomes available at a slow rate because rarely are patients referred to the Institute for removal of cataractous lenses. Detailed preoperative biomicroscopic examination and immediate fixation of the lens after intracapsular delivery are prerequisites for such a study. It is difficult, therefore, to obtain suitable preparations from other hospitals, but attempts will be made to develop such cooperative efforts since Dr. Wanko emphasizes the need of a large number of specimens for systematic analysis of such lenses.

Electron microscopic studies on the protozoon, Toxoplasma gondii, and of Toxoplasma cysts obtained from the peritoneal exudate of infected mice have been reported from several laboratories. Dr. Wanko could compare the electron micrographs of such material with those of cysts obtained by micromanipulation and in sections of brain tissue. The latter material provided important information on the development of the cyst wall which in its inner part seems to derive from the organisms, whereas the outer portion appears to be produced by the host cell.

In the electron microscopic studies on biopsy material of human muscular diseases great progress has been made as the findings could be included in comprehensive studies of Dr. Shy on two distinct disease entities, that is, of Familial Periodic Paralysis and Central Core Disease. There is no doubt that the electron microscopic findings represent valuable contributions to the pathomorphology of a rare disease in which the extent of pathological changes varied with the clinical state of the disease and to the cytology of a new clinicopathological syndrome. To avoid overlapping of reports, the description of the observed changes are omitted in this summary.

Among clinical investigations of this Branch the Glaucoma Study has made the greatest progress. This is partly due to the growing number of methods developed in the past years for investigating early phases of the disease, but even more so to the profound interest in this problem, the insight and talent of Dr. E. Okun in inspiring and organizing work around specific questions. Such questions concerned the reproducibility of tonographic recording, evaluation of provocative tests and combination of such tests, the diagnostic value of diurnal changes of intraocular pressure and outflow values, and the nature of the disturbed aqueous dynamics in various forms of glaucoma. Many new leads are contained in the recorded results of the extremely time-consuming examinations. They have to be confirmed by additional observations, by examinations repeated after long periods of time, and by continued studies of therapeutic effects. Then the observation of the accumulated material will be an invaluable source of information for a better understanding of the glaucomatous process and the rational of therapeutic procedures.

In contrast with the glaucoma project, little new knowledge was gained on clinical and laboratory studies of patients with uveitis, particularly those forms probably of Toxoplasmic nature. The patients admitted on this project stayed a total number of days which surpassed that of all other projects put together, and the referrals of patients suffering from uveitis outnumber by far all other requests. The investigative work in many

ophthalmological departments throughout the country continues to move along the outside fringes of the problem without approaching its core. The results of diagnostic procedures are at best suggestive and the effect of therapy is difficult to evaluate since individual attacks are usually self-limited and the specificity of "etiologic" treatment is questionable in patients who enjoy the excellent medical care of this hospital. A comparison of a modified hemagglutination test with the dye test, a study of the questionable specificity of treatment with Daraprim and sulphonamides, and the evaluation of steroid treatment in certain forms of the disease are under consideration.

On the ERG project psychophysical testing of patients with retinal diseases of various natures is being continued and referral of patients to the Branch for such testing from other medical centers is still frequent. The diagnostic potentialities of the ERG combined with adaptometry and color sense studies promises to have a new impetus in the near future in view of Dr. Gouras's interest in this problem and his competence in studying electrophysiological events. To which extent patients admitted to this Branch were used as subjects for basic studies of retinal pigments has been outlined in the summary of Dr. Rushton's work.

Cooperative endeavors of Dr. Schwab of the National Cancer Institute and Dr. Okun of this Branch resulted in an interesting study of the reversal of the retinopathy in Waldenstrom's Macroglobulinemia by Plasmapheresis. For the first time it was shown convincingly that the high serum macroglobulinemia and high serum viscosity are causatively connected with the clinical picture of the retinopathy. The fundus signs decreased or disappeared with plasmapheresis therapy, when it resulted in a fall of serum viscosity and the macroglobulin level.

Patients of the Heart Institute who were treated with 1-phenyl-2-Hydrazinopropane (JB-516) developed ill-defined color defects and transient or permanent reduced visual acuity. Eight such patients examined in the Ophthalmology Branch were reviewed by Dr. Jones and the cause of the functional disturbances described. He added to these observations the histopathological report of a patient who came to autopsy. Signs of bilateral inflammatory and degenerative lesions were discovered in the optic tract, anterior to the lateral geniculate body. These pathologic changes may explain the presence of the central scotomata which have been plotted in the course of the disease.

Dr. B. Cohan and Dr. R. Oglesby submitted interesting case reports which were accepted for publication by leading ophthalmologic journals as they contain new and interesting observations. Dr. Cohan followed a patient whose main complaint was tearing of the eye. This symptom was proved to be produced by a primary epidermoid carcinoma of the nasopharynx which had extended into the lacrimal sac filling its lumen and replacing the mucosa lining. Dr. Oglesby observed in a patient with documented cryoglobulinemia and reticulohistiocytosis a bizarre opacity in the posterior portion of the cornea which might have been caused by a deposition of abnormal globulins in this layer. No other report of such a lesion has been found in a survey of pertinent literature.

The diversity of the investigative efforts in the various laboratories and in the clinical field of this Branch made it impossible to briefly draw the threads together in a continuous and simplified resume. A general statement applying to all areas can be made however, that the results obtained in some completed investigations and the findings of studies in progress are believed to advance our knowledge in many directions, as these studies were conducted by critical investigators and competent observers. Whatever has been achieved is by no means to the credit of the principal investigator alone, but in no small part to the credit of co-workers, associates, research assistants, technicians, and indirectly to the dedication of the nursing, secretarial and clerical staff.

PROJECTS
Ophthalmology Branch 1960

Visual Processes

Rushton, W. A. H.: Dark Adaptation and the Regeneration of Rhodopsin

Rushton, W. A. H., Fuortes, M. G. F., Gunkel, R. D.: Increment Thresholds in a Subject Deficient in Cone Vision.

Rushton, W. A. H., Fuortes, M. G. F., Gunkel, R. D.: Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision.

Fuortes, M. G. F.: Studies on the Activity of Cells in the Eye of the Limulus.

Tansley, K., Copenhaver, R. M., Gunkel, R. D.: Some Observations on the Off-Effect of Mammalian Cone Electroretinogram.

Tansley, K., Copenhaver, R. M., Gunkel, R. D.: Some Aspects of Electro-retinographic Responses in the American Red Squirrel.

Tansley, K., Copenhaver, R. M., Gunkel, R. D.: Investigations on Spectral Sensitivity of Diurnal Squirrels. (ERG Reactions of Pure Cone Mammalian Retinae.)

Gouras, P.: Organizational Aspects of Vertebrate Retina Determined Electrophysiologically.

Gouras, P., Gunkel, R. D., Bonting, S. L., Caravaggio, L.: Electro-physiologically and Photochemical Relationships in the Vertebrate Retina.

*
Bonting, S. L., Caravaggio, L. L., Gouras, P.: The Rhodopsin Cycle in the Developing Mammalian Eye.

Gunkel, R. D.: Design and Construction of Ophthalmic Instruments.

Biochemical

Bonting, S. L., Simon, K. A.: Membrane ATP-ase and Aqueous Formation.

Resnik, R. A., Papaconstantinou, J.: A Study of the Proteins of the Lens.

Resnik, R. A., Trams, E. G., Irwin, R.: The Structure of Ganglioside.

Intraocular Pressure and Pharmacology

van Alphen, G., Robinette, S.: Basic Factors in Refraction Anomalies.

*Gouras, P.: The Adaptation of Electrical Enhancement Techniques to Clinical Electroretinography.

Intraocular Pressure and Pharmacology (Continued)

Macri, F. J.: Study on the Pharmacodynamics of Various Agents Affecting Intraocular Pressure.

Macri, F. J.: The Role of the Vasculature in the Maintenance of Intraocular Pressure.

Scullica, L., Macri, F. J.: Observation of the Flow Pattern in the Ciliary Processes of Albino Rabbits. An in vitro study.

Cytology

von Sallmann, L., Grimes, P., Collins, E. M.: Studies of the Effect of High Energy Proton Microbeams, Simulating Cosmic Primaries, on the Mouse Lens.

von Sallmann, L., Caravaggio, L. L., Grimes, P.: The Study on Mitosis in the Corneal Endothelium.

von Sallmann, L., Grimes, P., Weaver, K.: Studies of the Incorporation of H^3 Thymidine in the Corneal Endothelium of the Rabbit.

von Sallmann, L., Caravaggio, L. L., Collins, E. M., Weaver, K.: Effects of Chronic Administration of Corticosteroids on the Lens.

Wanko, T., Jacobs, L., Remington, J.: Electron Microscope Study on the Protozoon Toxoplasma gondii.

Wanko, T., Gavin, M. A., von Sallmann, L.: Electron Microscopic Studies on Tissues of the Eye, such as Epithelium, Fibers and Capsule of the Lens, Epithelium of the Ciliary Body, the Optic Nerve and the Conjunctiva.

Wanko, T., Shy, G. M., Gavin., M. A.: Electron Microscope Studies on Biopsies of Human Muscle Disease.

Huckel, Hubert: Influence of Alpha Chymotrypsin on the Healing of Corneal Endothelial Wounds.

Clinical Studies

Okun, E., von Sallmann, L., Jones, O., Oglesby, R., Simon, K.: Clinical Glaucoma Study.

von Sallmann, L., van Alphen, G., Okun, E., Oglesby, R. B., Simon, K., Jones, O., Huckel, H.: Study of Ocular Toxoplasmosis and its Therapy.

Jones, O. W., Gunkel, R. D.: Functional Studies in Retinal Anomalies and Diseases (Electroretinography, Adaptometry, and Perimetric Light Sense Studies.)

Clinical Studies (Continued)

Jones, O. W., Collins, E. M.: The Ocular Toxicity of 1-phenyl-2-Hydrazino-propane (JB-516.)

Oglesby, R. B., von Sallmann, L., Black, R. L., Bunim, J. J.: Corticosteroids as a Cause of Cataract in the Human.

Case Reports

Cohan, B. E.: Epiphora and Nasopharyngeal Tumor.

Oglesby, R. B.: Corneal Opacities in a Patient with Cryoglobulinemia and Reticulohistiocytosis.

PUBLICATIONS*
Ophthalmology Branch
1960

1. Cohan, B. E.: Epiphora as the Initial Manifestation of a Malignant Nasopharyngeal Tumor. Am. J. Ophth., 50:644-649, 1960.

2. Frank, K., Fuortes, M. G. F.: Accommodation of Spinal Motoneurones of Cats. Arch. Ital. Biol.,98:165-170, 1960.

3. Fuortes, M. G. F.: Inhibition in Limulus Eye. From "Inhibitions of the Nervous System and y-Aminobutyric Acid." Pergamon Press, New York, 418-423, 1960.

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5. Kaufman, H. E.: Uveitis Accompanied by a Positive Toxoplasma Dye Test. A. M. A. Arch. Ophth., 63:767-773, 1960.

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7. Macri, F. J.: Further Studies on the Action of Acetazolamide and the Venous Pressure of the Eye. A. M. A. Arch. Ophth., 64:550-552, 1960.

8. Macri, F. J.: Acetazolamide and the Venous Pressure of the Eye. A. M. A. Arch. Ophth., 63:953-965, 1960.

9. Macri, F. J.: Vascular Pressure Relationship and the Intraocular Pressure. A. M. A. Arch. Ophth. (In Press.)

10. Macri, F. J.: A Study of the Interdependence of Intraocular Venous and Eye Pressure. A. M. A. Arch. Ophth. (In Press.)

11. Black, R. L., Oglesby R. B., von Sallmann, L., Bunim, J. J.: Posterior Subcapsular Cataracts Induced by Corticosteroids in Patients with Rheumatoid Arthritis, J. A. M. A., 174:166-171, 1960.

12. Bloch, K. J., Wohl, M. J., Ship, I. I., Oglesby R. B., Bunim, J. J.: Sjogren's Syndrome I. Serologic Reactions in Patients with Sjogren's Syndrome with and without Rheumatoid Arthritis. Arthritis and Rheumatism, 3:287-297, 1960.

13. Oglesby, R. B.: Corneal Opacities in a Patient with Cryoglobulinemia and Reticulohistiocytosis. A. M. A. Arch. Ophth. (In Press.)

*Publications listed as "in press" in the report of 1959 are not included here, but are included in Part B of the appropriate projects.

14. Oglesby, R. B., Black, R. L., von Sallmann, L., Bunim, J. J.: Cataracts in Rheumatoid Arthritis Patients Treated with Corticosteroids (Description and Differential Diagnosis.) A. M. A. Arch. Ophth. (In Press.)

15. O'Rourke, J., Collins, E. M.: P-32 Localization of Malignant Melanoma of the Posterior Choroid. A. M. A. Arch. Ophth., 63:801-811, 1960.

16. O'Rourke, J.: A Syndrome of Hypometabolism and Depressed Thyroxine Utilization in Association with Uveitis. A. M. A. Arch. Ophth. (In Press.)

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18. Paton, D., Mclaren, D. S.: Bitot Spots, Am. J. Ophth., 50:568-574, 1960.

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21. Rushton, W. A. H.: The Intensity Factor in Vision. Symposium - Life and Light. The Johns Hopkins University Press (In Press.)

22. Rushton, W. A. H., Fuortes, M. G. F., Gunkel, R. D.: Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision. J. Physiol. (In Press.)

23. Rushton, W. A. H., Fuortes, M. G. F., Gunkel, R. D.: Increment Thresholds in a Subject Deficient in Cone Vision. J. Physiol. (In Press.)

24. Tansley, K., Copenhaver, R. M., Gunkel, R. D.: Spectral Sensitivity Curves of Diurnal Squirrels. J. Vision Research (In Press.)

25. Tansley, K., Copenhaver, R. M., Gunkel, R. D.: Some Observations on the Off-Effect of the Mammalian Cone Electroretinogram. J. Opt. Soc. Am. (In Press.)

26. Tansley, K., Copenhaver, R. M., Gunkel, R. D.: Some Aspects of the Electroretinographic Response of the American Red Squirrel, Tamiosciurus Hudsonicus Loquax. J. Cell. Comp. Physiol. (In Press.)

27. von Sallmann, L., Paton, D.: Hereditary Benign Intraepithelial Dyskeratosis. A. M. A. Arch. Ophth., 63:421-429, 1960.

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30. Wanko, T., Gavin, M. A.: Cell Surfaces in the Crystalline Lens. Symposium on the Structure of the Eye. Academic Press, New York, 221-223, (In Press.)

31. Shy, G. M., Wanko, T., Rowley, P. T., Engel, W. K.: Studies in Familial Periodic Paralysis. Experimental Neurology (In Press.)

Serial No. NINDB - 21 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effects of Chronic Administration of Cortico-steroids on the Lens

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Leo L. Caravaggio, M.S.
Eleanor M. Collins
Kirk Weaver

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.80
Professional: .20
Other: 1.60

Project Description:

Objectives: 1) to investigate the possible cataractogenic effect of chronically administered high doses of cortico-steroids on the lens of the rat. 2) to examine whether such effects exist on a cellular level. 3) to study the histopathology of lenses of patients who developed cataracts when treated for rheumatoid arthritis with high doses of steroids for long periods.

Methods Employed: Thirty-five male rats of the Sprague-Dawley strain weighing 100 to 200 grams received subcutaneously 15, 10 or 20 micrograms of Decadron. In a smaller group of animals the steroids were introduced by stomach tube. Control animals were injected with the vehicel. Food intake and weight gains were checked periodically and samples of blood for sugar determinations were obtained by heart puncture

at three-week intervals. Biomicroscopic examination of the lenses, weekly or bi-weekly were continued for 16 weeks at whichtime the experiments were terminated. The right eyes were prepared for histological examination the left eyes for flat mounts of lens epithelium and mitosis counts in this cell layer.

Major Findings Decreased weight gains of the differently treated animals when compared with each other and controls indicated the effectiveness of the steroid injections. The retardation of body growth was greatest in the group treated with the highest dose, but still significant in the animals which received five micrograms of Decadron. Biomicroscopic examination never revealed opacities or early signs of cataract formation which could be connected with the treatment. As a rule, the blood sugar in the experimental group did not differ from the controls and only a few animals had a transient elevation of the blood sugar level. Histological examination did not demonstrate any abnormalities in the architecture of the lens bow or in the delicate structures of the lens fibers. Other tissues of the eye also did not deviate from the normal The mitotic counts in the lens epithelium in the treated eyes were of the same magnitude as those of the controls. The alleged depressant effect of steroid therapy on cell division could not be demonstrated on the lens epithelium under the conditions of the experiment. Skin abcesses reported to occur at the site of injection were not observed in our series. Thus, the suggestion that these complications caused the decrease of weight gain could not be substantiated It was concluded that chronic administration of high doses of corticosteroids continued for four months does not affect the lens of the young growing rat when examined biomicroscopically, histologically and cytologically. Since the picture of rheumatoid arthritis or other collagen diseases cannot be produced experimentally, the study could not simulate the conditions existing in patients who developed lenticular opacities.

The microscopic examination of human material is presently limited to lenses of two patients who are considered to have steroid-induced cataracts. The examination has been completed in one patient who came to autopsy six hours after death. The lens epithelium mounted flat showed the normal mosaic of the central portion, regular equatorial rows and an undisturbed germinative zone. A comparatively small number of migrated bow nuclei were seen histologically whereas extensive cataractous changes were noted in the posterior cortex and less pronounced lesions in the anterior cortex of the lens. It is probable that the damage originates from a proteolytic process in the cytoplasm of the fibers near the posterior pole and extends from here into the superficial layers of the cortex. The migration of bow nuclei seems to be secondary to this lesion. As compared with senile cataracts of the posterior cortical type, the minor involvement of the germinative zone should be mentioned, but no clear-cut difference between the two types of cataract were established as yet. In contrast to this, the clinically similar radiogenic cataract differs histologically from the steroid induced one, as the lesion there seems to result from injury to the germinative zone of the lens epithelium.

Significance to Program of Institute: The evidence provided by clinical observations of patients strongly indicates that the slow development of lenticular opacities around the posterior pole is one of the undesirable side-effects of prolonged steroid treatment. To study the pathogenesis of this cataract might provide information on factors involved in the development of certain types of cataractous changes in the human and animal lens.

Proposed Course of Project: When lenses of patients who have been subjected to prolonged treatment with high doses of synthetic steroids become available, cytological, histological, microscopic and electron microscopic examinations will be carried out. Attempts will be made to reproduce the lesion experimentally in another species, (guinea pigs).

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Black, Roger L., Oglesby, Richard B., von Sallmann, Ludwig, and Bunim, Joseph J.: Posterior Subcapsular Cataracts Induced by Corticosteroids in Patients with Rheumatoid Arthritis. J.A.M.A. 174:166-171, 1960.

Oglesby, Richard B., Black, Roger L., von Sallmann, Ludwig, and Bunim, Joseph J.: Cataracts in Rheumatoid Arthritis Patients Treated with Corticosteroids. A.M.A. Arch. Ophth. (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 22 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of the Incorporation of H^3 Thymidine in the Corneal Endothelium of the Rabbit

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: Kirk Weaver

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.80
Professional: .20
Other: 1.60

Project Description:

Objectives: 1) to study mitotic activity and replacement rate in the corneal endothelium by labelling nuclei with H^3 thymidine in the synthetic phase of cell division by prolonged administration of the radioactive material. 2) to investigate migration of labelled nuclei in the process of growth and replacement.

Methods Employed: In preliminary studies, various techniques were used to allow chronic administration of chemical agents into the anterior chamber at a very slow rate of speed (0.5 ml. per 24 hours) without injuring and irritating the endothelium.

Fine polyethylene tubes, measuring from 100-300 microns in diameter, were introduced into the anterior chamber of the rabbit at

the limbus and fixed in position by properly placed sutures. The tubes coursed extraocularly underneath the conjunctiva and from the subconjunctival space subcutaneously to the back of the animal where they were anchored to the skin between the shoulder blades. When the postoperative reactions in the anterior chamber had subsided, an osmotic pump developed by Dr. Samuel Rose of NIAMD was attached to the tube and desired volumes of the substrate administered four times varying from a few hours to one-and-a-half days. During this period, arrangements were made that the animal rested comfortably and safely, so that it could not interrupt the flow in the tube. A mock operation was performed in the second eye which severed to compare the results obtained with those in the slowly perfused eye. The technique has many pitfalls which still have to be ironed out. The flat mount technique of the endothelium as described in another study was suitable for preparing whole mounts with endothelial side up and good radioautographs were obtained from a few of these preparations. Mr. William Matthews of the Medical Neurology Branch made the autographs with the stripping film technique.

Major Findings: The study is in a preliminary state. It was shown, however, that 1) the number of labelled nuclei seem to exceed the number of cells which were calculated to pass through mitosis during a day. 2) the labelled nuclei were abundant in small areas which appear to have been injured, but incorporation of the isotope was also seen at a great distance from the injury. 3) In the normal endothelium, labelling seems to take place in addition to the peripheral ring zone of the cornea in the central portion where dividing cells are usually absent.

Significance to Program of Institute: Cell proliferation and cell division of the corneal endothelium has been an unsolved problem in the past. It is desirable to learn more of the metabolism of this important cell layer which has the function to prevent the entrance of aqueous humor into the cornea. Whatever could be learned about the behavior of these cells under normal conditions, during aging and under pathologic situations might provide a basis for approaching clinical problems.

Proposed Course of Project: Considerable experience is still to be gathered to perfect the technique and to proceed with the work systematically. It might be possible to extend the study to other tissues of the eye

Part B included Yes [ ] No [X]

Serial No. NINDB - 23 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Study on Mitosis in the Corneal Endothelium

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Leo L. Caravaggio, M.S.
Patricia Grimes, B.A.

Cooperating Units None

Man Years (calendar year 1960): Patient Days: None
Total: 1.80
Professional: .20
Other: 1.60

Project Description:

Objectives: 1) to investigate the soundness of the generally held concept that mitosis does not take place in the endothelium of the cornea. 2) to study diurnal changes of mitotic activity and to determine the mitotic index of the endothelium of the growing animal.

Methods Employed: For studies on mitotic activity in the corneal endothelium and the role it plays in post-natal life, flat mounts are required which contain the whole cell population. A suitable technique for reliably providing whole mounts of the single cell layer attached to Descemet's membrane was developed in this laboratory.

The corneae of rabbits of various age groups (2-, 4-, 8-, and 12-weeks, 1 and 2½-years-old) were processed for the Feulgen reaction. Mitotic counts were obtained from both eyes of more than 60 animals.

Major Findings: In contrast to the generally held opinion that mitosis is absent in the endothelium except shortly after birth and that amitosis provides for tissue growth, it was demonstrated in the present study that mitotic activity exists in the endothelium of growing animals. The dividing cells are more numerous in the peripheral zone of the cornea than in the center. Their number decreases with age. To determine the mitotic index, it was necessary to calculate the cell population for each group. This was achieved by counting the cell nuclei per microscopic field and planimetric measurements of the whole area of the mount. The demonstrated mitotic activity of the corneal endothelium did not approach a value necessary for explaining the increase of size of the cornea by cell division alone. The cell counts in microscopic fields of different age groups and the measurements of the length axis of the endothelial nuclei supported the thesis that increase in cell size, and flattening of the cell body greatly contribute to the growth process. Diurnal variations of cell division were demonstrated by early morning and afternoon counts. The ratio of these two counts increased with age from 3.5 to 7.5

It was concluded that after growth has stopped, the corneal endothelium behaves as a non-renewal area where dividing cells in the fully grown animal are few or missing. These findings places the corneal endothelium in the group of tissues of which the vascular endothelium and the endothelium of the peritoneal cavity are the best examples.

Significance to Program of Institute: Studies on corneal endothelium, greatly neglected in the past, gain in clinical importance with the progress in corneal surgery. The knowledge of basic factors of cell proliferation in this tissue is a prerequisite for studying regeneration and pathological processes.

Proposed Course of Project: The newly developed technique of endothelial flat mounts opens several avenues which will be followed in studies on aging of the endothelium and on influencing regeneration potentialities.

Part B included Yes ☐ No ☒

Serial No. NINDB - 24 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of the Effect of High Energy Proton Microbeams, Simulating Cosmic Primaries, on the Mouse Lens

Principal Investigators: Dr. Ludwig von Sallmann
Dr. Howard J. Curtis

Other Investigators: Patricia Grimes, B.A.
Eleanor M. Collins

Cooperating Units: Brookhaven National Laboratory

Man Years (calendar year 1960): Patient Days: None
Total: 1.80
Professional: .20
Other: 1.60

Project Description:

Objectives: 1) to study the cataractogenic effect of cosmic ray primaries on the mouse lens by a high energy proton beam, simulating the ionization patterns of cosmic primaries. 2) to establish the relation between impact areas of the beams of various diameters, the threshold dose and latency period for producing a radiogenic lesion in the lens. 3) to investigate regenerative properties of the lens-tissue constituents in the case of minimal radiation-induced injury. 4) to correlate the radiobiological effect on the lens with that produced in the mouse brain with a similar technique.

Methods Employed: It is assumed that the biologic effect of cosmic ray primaries results from the dense cluster of secondary protons surrounding the cosmic ray alpha particle. Such a cluster is said to create a

core of ionization about 25 mu in diameter. Beams of this and larger diameters were obtained with a 22.5 MEV deutron beam from the 60 in. cyclotron of the Brookhaven National Laboratory. Dr. Howard J. Curtis, Director of the Biological Department, organized the study and performed the radiation of the mice The animals under light nembutal anesthesia were mounted on a board so that the limbus of one eye at 12 o'clock could be exposed to the beam. The other eye served as control. Round apertures with diameters of 25, 75 and 1000 microns were placed in front of the target area to achieve the desired beam profile. The beam was relatively well collinated to a depth of 1.5 mm., the peak of the Bragg curve was expected to be found at the circumscribed site of the equatorial lens cortex. Doses from 1012 to 33,000 rad were used. The animals were studied biomicroscopically in bi-weekly intervals and killed at varying times for the histological examination. Cytological examination of flat mounts of the epithelium was carried out in a small portion of the series which, at present, comprises both eyes of 130 mice. Thirty-two mice were set aside to study late effects In this group six months have elapsed since the exposure to the proton beam.

Major Findings: Biomicroscopically visible opacities resembling fine, round dots or elongated or irregular shapes in the middle superior sector of the posterior cortex were observed in a small group of mice exposed to a 25 micron beam of 33,000 rads 20 weeks after radiation. The lesions were minimal and did not seem to progress in the following months. Occasionally, a similar, also minimal and even questionable injury was noted with lower doses. Irradiation with a 75 micron beam at a dose level of 5200 to 13,000 rads produced signs of radiation injury more frequently and at a shorter latency period then in the previous group. Lower doses caused minute changes five to six months after radiation. Within six months the lesion did not progress in this group. With the 1 mm. aperture dose of 5200 rads, fine opacities were produced in the 20-22 week period. Subsequently the number of these opacities increased, and in several instances were accompanied by diffuse, cortical opacification around the posterior pole. Similar signs of radiation damage was observed with doses of 2600 and 1012 rads

As expected the histology of the radiated eyes did not always parallel the results of biomicroscopic studies. In the group irradiated through the 1 mm. aperture, migrated, and degenerate bow nuclei and swollen fibers were seen as early as four weeks after exposure to 4700 rads or more. With the 25 microbeam such changes occurred at higher doses (13,000 and 33,000) at the 12 to 14 week interval. At the six-months interval, irradiation with 1012 rads through the 1 mm. aperture induced cataractous changes in all animals, whereas 33 times higher doses were necessary to give rise to occasional minute lesions in the lens when the apertures of 25 and 75 microns were selected.

A similar relation of doses and impact area on the one side and biologic effect on the other were observed in the brain as reported from the Brookhaven National Laboratory. These experiments, however, were terminated after six weeks and the doses exceeded by far those studied in

the present series. The assumption that, in a larger radiation area of the brain, vascular disturbances mediates the tissue damage is not applicable to the situation in the avascular lenses. On the basis of observations on the mouse lens it can be assumed that the biological effect of the simulated cosmic primaries are of a low order for this organ. The question of recovery from minimal radiogenic lesions in the lens and restoration to histological integrity needs further study.

Significance to Program of Institute: The comparison of biologic effects of proton microbeams on the lens, on avascular tissue and brain is obviously of great practical interest and has definite implications for basic radiobiology. There is a decreased sensitivity for small exposure volumes and the biological effect of primary cosmic rays may not be as damaging as it is thought to be.

Proposed Course of Project: Investigations of dose and area dependency of irradiation with high energy microbeams will be continued in long term experiments.

Part B included Yes [ ] No [X]

Serial No. NINDB - 25 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-102 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of Ocular Toxoplasmosis and its Therapy

Principal Investigators: Ludwig von Sallmann, M.D.
Gerard van Alphen, M.D.

Other Investigators: Edward Okun, M.D.
Richard B. Oglesby, M.D.
Kenneth Simon, M.D.
Ottiwell Jones, M.D.
Hubert Huckel, M.D.

Cooperating Units: None

Man Years (Calendar Year 1960): Patient Days: 4421
Total: 1.80
Professional: .20
Other: 1.60

Project Description:

Objectives: Evaluation of diagnostic procedures and the effectiveness of chemo-- and steroid therapy in patients with uveitis of possible toxoplasmic nature.

Methods Employed: The etiologic work-up on patients with uveitis consisted of a routine hemogram, urinalysis, sedimentation rate, x-ray of the chest and skull and often the hands, antistreptolysin O titer, brucella and tularemia agglutination test, toxoplasma dye test, serological tests for syphilis, measurements of the total protein and albumin and globulin fractions, Bentonite Flocculation test, blood sugar, blood urea nitrogen and serum calcium. Skin tests with toxoplasmin and toxoplasmin control, histoplasmin, coccidiodin, heterologous staphylococcal antigen, Dick test streptococcal antigen, and lymphogranuloma venerum, as well as the first and second strength purified protein derivative of tuberculin were also done. On many patients, samples of aqueous and serum were tested for precipitating antibodies or were used for virus cultures.

Consultants from NIAID and NIAMD cooperated in attempts to establish the etiological diagnosis.

Major Findings: Patients with uveitis referred to the National Institutes of Health comprise an "unfavorable" group, many of whom were resistant to previous therapy. Of those patients with definitely active uveitis, 43% showed a complete disappearance of activity after treatment with pyrimethamine (Daraprim) and sulfonamides.

The uveitis responding best to therapy with pyrimethamine and sulfonamides had begun before the age of 20, was unilateral and was not exclusively anterior. There was no correlation between the level of the dye test and the therapeutic responses.

The toxoplasmin skin test appeared to be an excellent screening test for antitoxoplasma antibodies and was positive in 95% of patients with positive toxoplasma dye tests who had not had steroid therapy immediately before skin testing. In only 1 patient 200 (an atopic patient reacting to all antigens) was the toxoplasmin control positive. However, in a recent series of patients, the toxoplasmin skin test was negative in several instances in patients who had a high toxoplasma dye test titer.

Significance to the Institute: Although the results with the use of toxoplasmacidal drugs are encouraging when they can be administered in a relatively acute stage of the disease and in patients which showed the first manifestation at an early age, the specificity of the treatment is not established.

Proposed Course of Project: Patients with uveitis and negative toxoplasmin skin tests and negative dye test titers will be treated with Daraprim and sulfonamide to determine a non-specific effect of this treatment. Patients who do not tolerate the therapy and respond with a severe depression of the platelet counts will be treated with Vitamin B-12 and folic acid.

Part B Included Yes /X/ No. /___/

Serial No. NINDB - 25 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Kaufman, H.E.: Uveitis Accompanied by a Positive Toxoplasma Dye Test, A.M.A. Arch. Ophth., 63:767-773, 1960

Honors and Awards relating to this project: None

Serial No. NINDB - 26 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-78(c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Basic Factors in Refraction Anomalies

Principal Investigator: Gerard van Alphen, M.D.

Other Investigator: Sarah Robinette, B.S.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2.0
Professional: 1.0
Other: 1.0

Patient Days: None

Project Description:

Objectives: To complete a theory on the origin of refraction anomalies with particular reference to the role of the ciliary muscle and choroid.

Methods:

1) Physiological: The effects of sympathetic and parasympathetic stimulation on the pressure in the suprachoroidal space and the tension of the choroid (as determined by direct strain gauge measurements) were extended to studies of the elasticity of the sclera. In these experiments, strain gauges were directly placed on the sclera, at the equator and at the posterior pole, of isolated perfused cat eyes at constant IOP pressure.

2) Pharmacological: Strips of ciliary muscle, choroid, and ciliary muscle--choroid preparations were suspended in a muscle bath. The effects of cholinergic and adrenergic agents on the tension of the strips was recorded. Under similar conditions, strips of iris were examined in order to determine the effects of various pharmacological agents on the isolated sphincter and dilator muscle.

Major Findings: Although the scleral elasticity is clearly influenced by parasympathetic stimulation as a result of contraction of the ciliary muscle-choroid layer, the effect of sympathetic stimulation remained doubtful. This agrees with earlier experiments where sympathetic stimulation had conjectural effects on the pressure in the suprachoroidal space. Yet isolated strips of ciliary muscle and ciliary muscle-choroid combinations revealed a clear-cut relaxation on adrenergic compounds. Under these conditions, cholinergic agents produced a contraction, the magnitude of which was dependent on the tensionin the muscle strip. Significantly, low tensions produced small contractions. Drug effects were also investigated on ciliary muscle choroid strips of other representatives of the vertebrates, as well as on their iris sphincter and dilator muscles. The sphincter appeared to contract on both cholinergic and adrenergic compounds while the dilator responded only to adrenergic compounds.

Significance to Program of Institute: As Steiger's theory on the origin of refraction anomalies, although widely adhered to, is obviously untenable - no adequate explanation exists for the mechanism of emmetropia or ametropia. A theory now proposed is based on two assumptions. The first is that the choroid counteracts part of the intraocular pressure and that the choroidal tension is one of the parameters which determines the final size of the eye. The second, that emmetropization is achieved and controlled by a feed-back mechanism from the macula to the ciliary muscle. These assumptions readily explain the statistical data on the optical elements of the human eye and are supported by physiological, pharmacological and psychological evidence.

Proposed Course of Project: This project is terminated and will be submitted for publication. As far as the factor analytic part is concerned, it would seem that a further analysis may reduce the statistical data to just two basic factors, since two of the three factors reveal a rather marked correlation. A new project will be taken up to determine whether or not the diminished contraction on cholinergic agents of isolated ciliary muscle strips under low tension is of essential significance in glaucoma since it may be assumed that the continuous growth of the lens will relax the ciliary muscle. This not only will reduce outflow, but may also induce a critical bloodflow to the circle of Zinn which supplies the optic nerve head.

Part B Included: Yes [ ] No. [X]

Serial No. NINDB - 27 (c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Ocular Toxicity of 1-phenyl-2-Hydrazinopropane (JB-516)

Principal Investigator: O.W. Jones, III, M.D.

Other Investigators: Eleanor M. Collins

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3.0
Professional: 1.0
Other: 2.0

Project Description:

Objectives: To determine and evaluate the ocular toxicity of JB-516 (1-phenyl-2-hydrazinopropane) in patients being treated with the drug for hypertension.

Methods Employed: Patients being treated with JB-516 were periodically given complete ophthalmological examinations which included visual acuity, biomicroscopic examination, funduscopic examination, visual field examination, and color vision testing with the Hardy-Rand-Rittler Pseudoisochromatic plates and the Farnsworth D-15 Panel.

Autopsy tissue was available from one patient, which included the posterior half of the right globe, both optic nerves, the optic chiasm, the optic tracts, the lateral geniculate bodies, and the calcarene cortex. These tissues were fixed in 10% formalin and embedded in parafin and celloidin. The following staining techniques were used:

Sudan IV and oil red O (frozen section), hematoxylin and eosin, Luxol fast blue and cresyl fast violet and bodian.

Major Findings: Of the eight patients studied, all eight developed a decrease in visual acuity and a red-green color defect. Two patients developed central scotomata in addition. Six patients recovered after cessation of the drug. The two patients with the central scotomata did not recover normal vision and one of the two patients died and a bilateral optic neuritis was present in each optic tract just anterior to the lateral geniculate bodies.

Both clinical and pathological evidence demonstrated optic neuritis caused by JB-516

Significance to Program of Institute: To further the knowledge of the causes of toxic optic neuritis.

Proposed Course of Project: Project is terminated.

Part B included Yes [ ] No [X]

Serial No. NINDB - 28 (c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Clinical Investigations on Rare Diseases of the Eye
1. Epiphora and Nasopharyngeal Tumor
2. Corneal Opacities in a Patient with Cryoglobulinemia and Reticulohistiocytosis

Principal Investigators: 1. Bruce E. Cohan - 2. Richard B. Oglesby

Other Investigators: -----

Cooperating Units: National Institute of Arthritis and Metabolic Diseases.

Man Years (calendar year 1960): Patient Days: None
Total: 2.0
Professional: 2.0
Other: 0

Project Description - No. 1:

Objectives: To demonstrate the importance of a minor clinical sign for the diagnosis of a malignant disease in the tear sac.

Methods Employed: Routine ophthalmologic and histopathologic examinations.

Major Findings: A patient, later recognized as having a primary epidermoid carcinoma of the nasopharynx involving the lacrimal sac, presented himself with the symptom of epiphora. The extension of such a neoplasm to the lacrimal sac is extremely rare. Histologically the tumor filled the lumen of the sac and replaced its mucosa. It consisted of nests and cords of undifferentiated epithelial cells with indistinct cytoplasma limits between strands of connective tissue. On autopsy, metastases of the carcinoma were shown in the many organs.

Significance to the Institute: It is brought out by this observation that epiphora might be the first clinical manifestation of a neoplasm in the tear sac.

Proposed Course of Project: Terminated.

Part B Included Yes /x/ No. /___/

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cohan, B. E.: Epiphora and Nasopharyngeal Tumor, Am. J. Ophth. 50:644-649, 1960.

Honors and Awards relating to this project: None

Project Description - No. 2:

Objectives: To describe and evaluate the ocular abnormalities in a patient with documented cryoglobulinemia and reticulohistiocytosis.

Methods Employed: Routine clinical and ophthalmological examination, routine chemical, hematological and serological studies of blood, serum electrophoresis and ultracentrifugation, biopsy histology, electronic tonography, medical illustration.

Major Findings: Ocular abnormalities were found in the cornea in the form of discrete opacities in the posterior fourth, which spared the limbal and central areas, and in the lens, which displayed posterior subcapsular cataracts. Scleral rigidity and aqueous outflow were normal. Corneal opacities were not found reported in either of these diseases in a survey of pertinent literature.

Significance to Program of Institute: Inasmuch as both abnormal protein and abnormal lipid have been shown in the tissues from skin and joint of this patient, it can be conjectured that the corneal opacities are caused by one or both of these substances.

Proposed Course of Project: Other patients with abnormal proteins and/or lipids will be studied as they become available.

Part B Included Yes [X] No. [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Oglesby, R. B.: Corneal Opacities in a Patient with Cryoglobulinemia and Reticulohistiocytosis, A.M.A. Arch. Ophth. (in press).

Honors and Awards relating to this project: None

Serial No. NINDB - 29 (c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Corticosteroids as a Cause of Cataract in the Human

Principal Investigator: Richard B. Oglesby, M.D.
Ludwig von Sallmann, M.D.

Other Investigators: Roger L. Black, M.D.
Joseph J. Bunim, M.D.

Cooperating Units: National Institute of Arthritis and Metabolic Diseases
Medical Arts

Man Years (calendar year 1960): Patient Days: None
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To delineate the incidence, morphology, visual effects and course of cataracts observed in patients on long-term, high dose corticosteroid therapy.

Methods Employed: Detailed ophthalmological survey of all rheumatoid arthritis patients admitted to NIH, with similar examination of other patients on corticosteroid therapy as well as an untreated control group. Medical evaluation of patients for other causes of cataract. Medical illustration of selected cases.

Major Findings: Seventeen of 44 (39%) patients with rheumatoid arthritis on prolonged corticosteroid therapy had posterior subcapsular cataracts and 19 controls, untreated with corticosteroids, did not have these cataracts. The occurrence of these cataracts was correlated with dosage and duration of corticosteroid therapy, and not with age of patient, severity or duration of rheumatoid arthritis, other therapy, or systemic disease. Patients with psoriasis, lupus erythematosis, periarteritis nodosa, and dermato myositis treated with corticosteroids have also been observed to develop these cataracts.

Visual effects have been serious in only a few cases thus far.

Significance to Program of Institute: Posterior subcapsular cataracts are reported as a new side effect of corticosteroid therapy. Clinically, this emphasizes the necessity of limiting both dose and duration of such therapy to an absolute minimum.

Proposed Course of Project: Further clinical observation and follow-up are in progress.

Part B Included Yes /X/ No. /___/

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Black, R.L., Oglesby, R.B., von Sallmann, L., Bunim, J.J.: Posterior Subcapsular Cataracts Induced by Corticosteroids in Patients with Rheumatoid Arthritis, J.A.M.A., 174:166-171, 1960.

Oglesby, R.B., Black, R.L., von Sallmann, L., Bunim, J.J.: Cataracts in Rheumatoid Arthritis Patients Treated with Corticosteroids, A.M.A. Arch. Ophth., (submitted for publication), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 30 (c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland
4. Same as NINDB-80 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Clinical Glaucoma Study

Principal Investigators: Edward Okun, M.D.
Ludwig von Sallmann, M.D.

Other Investigators: Ottiwell Jones, III, M.D.
Richard Oglesby, M.D.
Kenneth Simon, M.D.

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: 1794
Total: 4.0
Professional: 4.0
Other: 0

Project Description:

Objectives:

1) The main objective is to evaluate suspected and known cases of glaucoma in an effort to find earlier means of diagnosis and better means of control.

a) The reliability of tonography as a diagnostic tool is being critically evaluated.

b) Provocative tests utilizing tonography and applanation tonometry are evaluated.

c) The mechanism of diurnal changes in intraocular pressure is being investigated by means of tonography.

d) The mechanism of action of various changes and operative procedures is studied by tonography and applanation tonometry.

2) To further investigate the disturbed introocular fluid dynamics in glaucoma, the inflow mechanisms are studied. Just as tonography gives an indication of the ease with which fluid leaves the eye, the inflow studies give an indication of the rate with which fluid enters the eye.

Methods Employed: Each patient acceptable for the study is admitted to the hospital for a minimum of several days in order that an extensive glaucoma work-up can be performed. In addition to the history and general physical examination, specific tests include tonometry with day curve, applanation tonometry, biomicroscopy, gonioscopy, repeated tonography with and without provocative tests, and studies of recovery from tonography as determined with the applanation tonometer. In addition, visual field studies are carefully performed using the Goldmann perimeter and Gunkel tangent screen. When the patient's intraocular fluid dynamics have been observed over a time interval, the response to indicated therapy is subsequently evaluated. Patients are followed at four to six month intervals after discharge, at which time they are re-evaluated.

Major Findings: At the present time, the number of patients in this study is too small, and their period of follow-up too short to draw many conclusions from this long-term project. Although the study on tonography repeatability is not yet completed, it appears that tonography, when properly performed, gives very reliable results. It has proven to be of great value in conjunction with dilatation provocative testing for narrow angle glaucoma. At the present time, the cases of borderline glaucoma with positive water drinking provocative tests have not been followed for a sufficient period of time to judge the value of the data obtained. Studies of the inflow mechanisms by means of applanation tonometry are not yet completed, but there are indications that this may be a fairly sensitive means of measuring inflow.

Significance to Program of Institute: The described project is of timely importance to the Public Health Service due to the growing awareness of doctors and laymen alike concerning the significance of early glaucoma diagnosis. Though clinical experiments with glaucoma, laboratory investigations are further inspired. It is emphasized that this Institute offers a unique opportunity for such detailed observations of glaucoma patients, for routine laboratory studies can be repeatedly performed under a considerably longer period of hospitalization than is available to private institutions. Through the quality of prolonged study, rather than through the quantity of patients examined, this project should offer information which cannot be readily gained elsewhere.

Proposed Course of Project: It is hoped that the size of this project will continue to increase. A reservoir of well-studied patients who reside in the Washington area is being built up and it is thought that by continuing studies on this increasing population a means of earlier diagnosis can be established. The next logical step will be the evaluation of various means to delay the onset of manifest glaucoma.

Part B Included Yes /___/ No. /X/

Serial No. NINDB - 31 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Activity of Cells in the Eye of the Limulus

Principal Investigator: Dr. M.G.F. Fuortes

Other Investigators: None

Cooperating Units: Marine Biological Laboratory
Woods Hole, Massachusetts

Man Years (calendar year 1960):
Total: 3.0
Professional: 1.0
Other: 2.0

Project Description:

Objectives: To study the changes evoked by light in the eye of invertebrates in various stages of adaptation.

Methods Employed: As in previous work glass micropipettes filled with three molar KCL were used for intracellular recording.

Major Findings: In conditions of dark adaptation, depolarizing transients can be recorded from cells of the limulus eye following application of weak lights (of the order of 10^4 quanta impinging upon the lens in one second). A statistical analysis of the relations between frequency of appearance of these transient potentials and light intensity has been performed. The results obtained are consistent with the hypothesis that absorption of one quantum may be sufficient for generation of one transient but other interpretations cannot be excluded.

The time course of dark adaptation has been studied by recording the responses to brief flashes of light and of brief depolarizing pulses (both of fixed intensity) before and after application of a strong light. It was found that the response to the depolarizing current recovers within a few seconds from the termination of the adapting light, but the response to the testing light pulses recovers only very slowly.

The responses to given light and current pulses have been recorded before, during and after application of background illuminations of moderate intensity. It was found that the background illumination increases the response to the currents (as judged by number of discharge spikes) but decreases the responses to light.

If a strong light is applied and then suddenly decreased to a lower intensity (but not to complete darkness) the nerve cell recovers a large membrane potential and later depolarizes progressively to a steady value. At the same time, the strong discharge of impulses which was present during the strong illumination is first abolished and later starts again at moderate intensity. Electrical conductance is decreased during the transient hyperpolarizing phase.

If light is increased by successive steps (e.g. light intensity 5, 25, 625, etc.), roughly equal increments in the response are recorded in steady state conditions, but very different changes occur just after the transition. In most cells a large transient depolarization is evoked by only one of the steps.

If light is increased progressively so that the logarithm of light intensity is a linear function of time a transient depolarization may still occur when a certain light intensity is reached. The light intensity at which the response becomes obviously non-linear is not greatly affected by the rate of change of illumination (the slope of the intensity-time relation of the stimulus).

In some cells a non-linearity similar to that just described occurs also when light is progressively decreased

Whereas light always evokes depolarization of the nerve cell in normal conditions, if the cell is hyperpolarized by means of a steady current the effect of light may be reversed Thus cells hyperpolarized by currents may become further hyperpolarized by illumination. This effect could be demonstrated only occasionally and it is not possible to measure corresponding conductance changes.

Significance to Program of Institute: It is hoped that analysis of the records obtained during the summer in Woods Hole will lead to a coherent and perhaps useful interpretation of the results described.

Proposed Course of Project: Dr. Fuortes has terminated his appointment in September of this year to head a neurological institute in Milan. Since the organization of the new laboratory has not been completed, further plans of Dr. Fuortes, with respect to investigations in this field, are not known.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Fuortes, M. G. F.: Inhibition in Limulus Eye. From Inhibitions of The Nervous System and y-aminobutyric acid, pp. 418-423, 1960, Pergamon Press, New York.

Honors and Awards relating to this Project: None

Serial No. NINDB - 32 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Organizational Aspects of Vertebrate Retina Determined Electrophysiologically.

Principal Investigator: Peter Gouras, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: .40
Professional: .40
Other: 0

Project Description:

Objectives: To determine the coding of color and intensity by the vertebrate retina at the level of the ganglion cells. The initial objective is to determine the dependency of ganglion cell discharges on the rate of change of photic stimuli.

Methods Employed: The methods are conventional for recording single unit activity either intra- or extracellularly. The stimulus presentation is unique in rate dependency experiments where slow continuous changes are attainable by rotating analyzers in polarized light or by the use of sinusoidal wedges or glow modulator tubes.

Major Findings: The ganglion cells of amphibian retina are shown to be "differentiators", i.e., any photic stimulus can be substituted for another including ones differing in chromaticity without the ganglion cell detecting it if the substitution is slow enough. Ganglion cells discharging a flickering stimulus varying as the function $\cos^2$ wt, where wt is the rotation of a polaroid in radians/sec, trace different curves to different wave lengths when flicker fusion frequency is plotted against intensity. Long wave length stimuli produce steeper curves than short wave lengths suggesting that scotopic (rods) and photopic (cones) mechanisms interact on single ganglion cell with long wave length being more effective in determining CFF at high intensities and short wave

lengths at low intensities in a manner analogous to the Purkinje shift. Another surprising result is that at either low intensities or low flicker rates the curve may turn back on itself so that fusion results, not only when the stimulus is too fast, but when it is too slow.

Significance to Project of Institute: The way in which visual information is carried to higher centers from the retina is of more than academic interest. A number of recent investigations by means of either the stabilized retinal image, the "ganz feld" technique, or sinusoidal flicker have indicated in a subjective way the importance of rate of change to vision. It emphasizes the major importance of ocular tremor, blinks, and the extraocular muscles in facilitating the eye to see. The above study confirms the fact that in amphibian retina, such a mechanism can be objectively found at the ganglion cell level.

Proposed Course of Project: The plan is to carry this work into higher vertebrates such as cat and rabbit and on retina left in situ.

Part B Included Yes / / No. /X/

Serial No. NINDB - 33 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Electrophysiological and Photochemical Relationships in the Vertebrate Retina.

Principal Investigator: Peter Gouras, M.D.

Other Investigator: Ralph D. Gunkel, O.D.

Cooperating Units: Cell Biology Section, Ophthalmology Branch, NIH, under the direction of S. L. Bonting, Ph.D., with the assistance of L. Caravaggio, M.S.

Man Years (calendar year 1960):
Total: .55
Professional: .55
Other: 0

Patient Days: None

Project Description:

Objectives: To describe the coupling mechanism between the chemical change resulting from photochemical bleaching and the electrogenesis leading to vision.

Methods Employed: The instrumentation from the electrophysiological aspect involves the conventional ERG stimulating and recording techniques as well as microtechniques including micromanipulators, electrode pullers, microscopes, and stereotaxic equipment. The biochemical methodology is described under the project report of the cooperating unit.

Major Findings: The first study under this project determined the development of the ERG in albino rats from birth to maturity. The ERG is first detectable at 12 days and thereafter increases rapidly to the 21st day. Subsequent increase is much slower, although still apparent at the 47th day. The a- and b-waves appear to develop at similar rates. There is a very close correlation between the electrical development and the increase in rhodopsin concentration. ERG spectral sensitivity curves of the dark adapted eye are similar to the rhodopsin absorption curve confirming the view that this is the predominant photopigment responsible for the animal's vision.

Recent results of the second study of this project have revealed that it is possible to determine dark adaptation curves for the rat, using ERG criteria. Different degrees of bleaching exposures produce different dark adaptation curves analogous to the psychophysical curves obtained in man. Biochemical studies in parallel with this are expected to reveal the relationship between rhodopsin concentration and ERG threshold and show whether it is the sole rate determining step throughout the entire range of dark adaptation.

Significance to Program of Institute: The significance of information elucidating the mechanism in which light generates electrical activity is of major importance to the physiology of vision. The structure at which the initial electrogenesis occurs in the retina is undoubtedly the principal source for the ERG, a problem whose solution remains unknown despite the widespread investigations on and clinical usefulness of the response itself.

Proposed Course of Project: Similar studies on the development of the ERG are planned on other experimental animals, including rats with pigmentary degeneration and others treated with iodacetate and glutamate.

A more intensive study is planned utilizing microelectrode techniques in order to determine the site of electrogenesis in the retina. Intracellular recordings are now possible from the ganglion cells of intact mammalian retina as well as horizontal cells lying closer to the photoreceptors and further experimentation along these lines should reveal more concerning photoreceptor activity.

Part B Included Yes / / No. /X/

Serial No. NINDB - 34 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Adaptation of Electrical Enhancement Techniques to Clinical Electroretinography

Principal Investigator: Peter Gouras, M. D.

Other Investigator: Ralph D. Gunkel, O.D.

Cooperating Units: Psychology Department, Forest Glen Section, Walter Reed Army Hospital, under the direction of John Armington, Ph.D.

Man Years (calendar year 1960): Patient Days: None
Total: .55
Professional: .55
Other: 0

Project Description:

Objectives and Methods Employed: In the last few years, electronic computer techniques have been finding increasing utility in biological research. One of the simplest applications of these techniques is in the field of electrophysiology when signal to noise discimination is a problem and the signal bears a fixed temporal relationship to the stimulus, i.e. evoked responses.

Electronic circuits when properly programmed can store the average of many thousands of responses to a fixed stimulus so that random signals eventually tend to cancel out and signals fixed to the stimulus sum. The signal to noise discrimination improves as the square root of the number of trials and electrical responses

as low as 0.5 uV become discernible. The ERG is particularly suited to such an approach. The responses presently detectable with conventional systems are seldom less than 25 uV., because of the noise from ocular, head, and body movements as well as electrical circuitry. The objective of this project is to develop an averaging system at NIH and to carry out the ERG studies which it makes possible.

For the past six months a workable unit has been in operation at the Psychology Department of Walter Reed under the direction of John Armington. For the past three months, we have been collaborating with this investigator on a number of projects, one of which is already at a publishable stage and will be described.

Major Findings: The study presently includes 10 patients with pigmentary degeneration, predominantly Retinitis Pigmentosa, who had "extinguished" ERG's on the conventional NIH recording system. All patients had responses with averaging, including some with only 5° central fields. There is a direct relationship between the functioning retina as estimated by clinical perimetry and the light energy necessary for producing a constant amplitude ERG. Spectral sensitivity curves determined on these subjects by this technique reveal predominantly photopic curves in patients with functioning macular retinae and more scotopic ones in those with more rod vision. The relationship between retinal area and ERG threshold in Retinitis Pigmentosa greatly weakens the Bruch's membrane "short circuiting" theory proposed to explain absent electrical responses in R.P. patients with only moderate retinal pathology. Such diseases can now be followed objectively into their most advanced stages and all subjective perimetric evaluations to determine progression can be confirmed and perhaps corrected electrically.

It has been routine to study the photically evoked responses from the occipital cortex during every study since such information is readily and simultaneously obtainable, using the same techniques. The responses are as easy to detect as the ERG and although somewhat dependent on the mental state of the patient, they have been instructive. We have recently shown a depression in the evoked cortical response in a subject having a migrainous scotoma, and we hope to follow this up by plotting the spatial spread of this depression by the use of both localized test stimuli and Laplacian electrodes.

Significance to Program of Institute: The development of a functioning averaging system for the ERG unit at NIH will enable it to be used as a diagnostic clinical tool as well as a springboard for further research into a field that has already been carried so far at NIH. The close liaison developing between our section and that at Walter Reed affords numerous mutual advantages. This group will soon be converting to a digital computer system, which provides greater resolution, higher frequency response and statistical evaluation of the data. The proximity of such an instrument will, of course, be of great assistance to the NIH program.

Proposed Course of Project: This project, begun in September 1960, will probably continue for at least the following calendar year. It is shortly expected to attempt the detection of localized retinal lesions using small test stimuli, another result hitherto unattainable with conventional recording systems. Objective retinal, as well as cortical perimetry, could be the outcome of such a program.

Part B included: Yes [ ] No [X]

Serial No. NINDB - 35 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-95 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Design and Construction of Ophthalmic Instruments

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: .50
Professional: .50
Other: 0

Project Description:

Objectives: To make improvements or modifications of instruments used in clinical and experimental ophthalmological work. Specifically, to design and/or construct such devices as are suggested or required by current projects and to follow through in their use.

Methods Employed: In addition to an unpredictable amount of work relating to clinical and psychophysical tests, this investigator has worked quite closely with Dr. Tansley and Dr. Rushton during their tenure here as visiting scientists. Numerous mechanical and optical devices have been produced to meet specific needs, thereby greatly facilitating the experimental work.

Many modifications were required for Dr. Rushton's densitometer in adapting it to the measurement of retinal pigments in different types of subjects. Instruments had to be made for measuring pupil

size, for bleaching the retina, for measuring increment thresholds, and for measuring the Stiles-Crawford effect.

The Goldmann adaptometer was further modified to facilitate its use in the study correlating dark adaptation with the regeneration of rhodopsin.

Major Findings: While it has not been possible for this investigator to pursue extensively his perimetric light sense studies, he has contributed substantially to the fruitfulness of the visiting scientists. Dr. Tansley's work, as reviewed elsewhere, has resulted in three papers relating to the histology and functioning of the pure cone retina. Dr. Rushton's work has resulted in three papers explaining certain aspects of retinal sensitivity, pigment bleaching and dark adaptation.

Significance to Program of Institute: The primary significance of this project appears to lie in the stimulation and encouragement the visiting scientists find in a person who can understand their technical problems and do something about them in spite of the unfamiliar equipment, procedures, and restrictions. In some cases it has also been very helpful to them to have an assistant who knows where and how to obtain certain types of subjects for experiments.

Proposed Course of Project: It seems likely that the next year can profitably be spent in collaborative work with Dr. Gouras. Preliminary experiments in collaboration with Dr. John Armington have demonstrated the feasibility of recording electroretinograms by electronic summation and averaging in patients with only five degree central visual fields. Heretofore, these cases have always been described as having extinguished ERG's. This suggests the exciting possibility of perimetric ERG studies and objective profiles of retinal sensitivity which would certainly be of great clinical interest. Not only would these studies be correlated with visual field measurements, but Dr. Gouras proposes a practical method for determining dark adaptation curves by a purely objective ERG method.

This union of laboratory and clinical procedures appears to follow closely the chief aims of the NIH.

Part B included Yes ☐ No ☒

Serial No. NINDB - 36 (c)

1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-96 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Functional Studies in Retinal Anomalies and Diseases (Electroretinography, Adaptometry, and Perimetric Light Sense Studies.)

Principal Investigators: O. W. Jones, III, M.D.
R. D. Gunkel, O.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.25
Professional: 1.25
Other: 0

Patient Days: 245
Outpatient Visits: 42

Project Description:

Objectives: This study is a continuation of a previous project. It is primarily concerned with the investigation of patients with congenital retinal anomalies and degenerative diseases of the retina. A comprehensive ophthalmological examination is combined with psychophysical testing such as adaptometry, electroretinography, and retinal profile studies. By this examination it is hoped to establish the differential diagnosis between ophthalmologically similar entities at an early state of development.

Methods Employed: After a complete routine ophthalmological examination, including careful field studies, the color vision is tested with the Hardy-Rand-Rittler pseudoisochromatic plates. The course of dark adaptation is determined on the Goldmann Adaptometer and a "retinal profile" is determined on Dr. Gunkel's modification of the Goldmann adaptometer. Electroretinography is performed on every patient. These ERG's are obtained by means of a contact lens electrode and recorded on the Grass 8-channel electroencephalograph recorder, or on an oscilloscope. The photopic and scotopic mechanisms are clearly distinguished by these techniques.

Major Findings: Many interesting and unusual cases were examined and studied. In most instances, the results of the special studies made it possible to give a correct diagnosis and to discuss prognostic aspects.

A few cases of achromatopsia were examined and significant electroretinographic data were obtained from these patients suffering from this rare condition. These patients were also used as subjects in studies of rhodopsin—bleaching and regeneration and rod-cone inhibition.

Significance to Program of Institute: The studies are conducted in an attempt to further our knowledge of the cause and progress of certain congenital and degenerative diseases of the retina and to subclassify the different disease entities.

Proposed Course of Project: Dr. Peter Gouras who has recently joined the Branch plans to extend these studies to better quantitate the results with an improved technique.

Part B Included Yes / / No. /X/

Serial No. NINDB - 37 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Increment Thresholds in a Subject Deficient in Cone Vision

Principal Investigators: W. A. H. Rushton, F.R.S.
M. G. F. Fuortes, M.D.

Other Investigators: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: 210
Total: .60
Professional: .60
Other: 0

Project Description:

Objectives: To investigate the rod vision of a patient with incomplete rod monochromacy and obtain information concerning normal rod function.

Methods Employed: The subject saw well in dim light. The contrasts disappeared and the field assumed a bright, undifferentiated character when the illumination increased. A retinal illumination of 1,000 trolands produced this effect and subsided within a second when illumination was reduced to 1 troland. It was not linked, therefore, with the bleaching and regeneration of rhodopsin. The vision was 20/80. The subject failed the usual color vision tests but good red and blue lights were correctly named.

An optical system was designed to project a small flashing light upon a background field and present it to the subject by Maxwellian view. A rotating sector in front of a zirconium lamp and an optical system interrupted the light so that a 1^{o} patch was on for half a second and off for half a second. With the aid of a fixation lamp it was possible to also measure parafoveal increment thresholds.

Major Findings: The subject appeared to have normal rod function and her rod increment threshold curve in the parafovea agreed with the results reported by Aguilar and Stiles as rod saturation was reached at 1,000 trolands. At this illumination increment perception was abolished and subjectively the patient lost the sense for visual contrasts.

Cone vision was restricted to the neighborhood of the fovea. The spectral sensitivity at this site was about normal and there was a Stiles-Crawford effect. The cone organization was not normal, however, because the threshold was extremely high, color vision almost absent, and visual acuity low. The spectral sensitivity of the saturating background was that of rhodopsin. On the basis of these observations the existence of a cone-rod inhibition in the normal is questioned.

Significance of Program to Institute: The study carried out on an incomplete rod monochromat has important implications on normal rod function and on interaction of cone and rod vision.

Proposed Course of Project: Dr. Rushton has terminated his appointment as a visiting scientist to this branch and it is not known in which direction he is going to continue the investigation in this area.

Part B Included Yes /X/ No. /__/

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Rushton, W. A. H., Fuortes, M. G. F., Gunkel, R. D.: Increment Thresholds in a Subject Deficient in Cone Vision, J. of Physiol., (in press).

Rushton, W. A. H.: Dark Adaptation and the Regeneration of Rhodopsin, J. of Physiol., (in press).

Rushton, W. A. H., Fuortes, M. G. F., Gunkel, R. D.: Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision, J. of Physiol., (in press).

Rushton, W. A. H.: The Intensity Factor in Vision, Symposium - Life and Light, The Johns Hopkins University Press, (in press).

Honors and Awards relating to this project: None

Serial No. NINDB- 38 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision

Principal Investigator: W.A.H. Rushton, F.R.S.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: 210
Total: 1.60
Professional: .80
Other: .80

Project Description:

Objectives: 1) to investigate in an incomplete monochromat (photanope) the visual pigments by retinal densitometer and the dark adaptation curve in relation to the amount of rhodopsin present. 2) to study in this patient the increment threshold as a function of background illumination.

Methods Employed: The apparatus, principles and the procedure of testing were similar to those previously described.

Major Findings: It was concluded that the photanope had about the normal amount of rhodopsin in the retina at 12° temporal to the fovea and that it regenerated at the normal rate. Attempts to measure any visual pigments in the photanope fovea were somewhat marred by irregular fixation. Within the limits of pigment detection under these circumstances, neither rhodopsin nor cone pigment could be detected in a 2° measuring, centrally fixated field.

In dark adaptation studies it was shown that in contrast to the normal subject, the photanope, not able to distinguish between yellow (580 mu) and green (520 mu), presented a smooth rod curve. The cone threshold had a castellated appearance, as these photoreceptors were 3 times more sensitive to yellow than to green. No Stiles-Crawford effect was detectable in the rod curve, whereas such an effect, although of low magnitude, was demonstrated in the cone branch. With respect to the great range of dark adaptation in the photanope, the possibility of scatter should be carefully excluded before classifying the receptors as "daylight" rods.

The situation in a rod monochromat permits the plotting of the rod dark adaptation curve also above the level of the cone threshold. It could be examined, therefore, whether there is an inhibitory effect of rods by cones. Comparing the dark adaptation curve of the normal with that of the photanope did not indicate an inhibition of the rods by cones but suggest the two visual receptors follow independent mechanisms.

In the second part of the study dealing with the relation between rhodopsin and threshold it was confirmed that log I/I_o in dark adaptation is proportional to the fraction of opsin uncombined with retinine. The results of the experiments of dark adaptation thresholds obtained against various background illumination provided a new basis to bring in line photochemical experimental data and results of studies on visual pigments in the human eye. For the time being doubt is expressed as to the tenability of Wald's compartment hypothesis of light adaptation which requires that a large fraction of incident quanta become entirely inoperative.

Significance to Program of Institute: The study provides most valuable information on the physiology of adaptation.

Proposed Course of Project: Dr. Rushton has terminated his appointment as a visiting scientist to this branch and it is not known in which direction he is going to continue the investigation in this area.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Rushton, W.A.H., Fuortes, M.G.F., Gunkel, R.D.: Increment Thresholds in a Subject Deficient in Cone Vision. J. of Physiol. (In Press), 1960.

Rushton, W.A.H.: Dark Adaptation and the Regeneration of Rhodopsin, J. of Physiol. (In Press), 1960.

Rushton, W.A.H., Fuortes, M.G.F., Gunkel, R.D.: Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision, J. of Physiol. (In Press), 1960.

Rushton, W.A.H.: The Intensity Factor in Vision, Symposium - Life and Light, The Johns Hopkins University Press (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 39 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Dark Adaptation and Regeneration of Rhodopsin

Principal Investigator: W. A. H. Rushton, F.R.S.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: 1.60
Professional: .80
Other: .80

Project Description:

Objectives: To determine the relationship between the threshold for light detection and the concentration of rhodopsin in the intact human eye.

Methods: The method consists in measuring the difference in the amount of light reflected back through the pupil from the fundus oculi before and after bleaching rhodopsin. The technique employs two beams of light traveling identical optical paths into the eye but oppositely polarized. One beam (516 mu) is maximally absorbed by rhodopsin and the other (deep red) is not absorbed. When an analyzer is moved in their common path the total light transmitted at any time can be represented by $r \sin^2 nt$ and $g \cos^2 nt$ where r and g are the intensities of the red and green beams respectively. Suitable adjustment of the intensity of one beam can make the output as well as the light reflected from the fundus constant over time. A change in the concentration of rhodopsin after a bright bleaching light will disturb

the balance which can be regained by readjustment of the neutral wedge, representing a double density change in retinal rhodopsin. The light reflected is measured by a photomultiplier, this output sent through a phase sensitive rectifier which compares it to another polarized signal changing at an identical rate, integrates the multiplied product (which is zero for all signals not changing at the rate the analyzer rotates and considered to be noise) and the final signal is measured by a galvanometer. Dark adaptation is measured by Gunkel's modification of the Goldmann-Weeker's adaptometer.

Major Findings: There appears to be a critical point at 90% rhodopsin regeneration at which the rods begin to make their contribution to threshold for light detection at the so-called rod-cone"kink" in the dark adaptation curve. It is argued that since both rod curves of dark adaptation obtained by different bleaching lights are superposable by lateral displacement along the time abscissa and that rhodopsin regeneration curves are likewise superposable on themselves that the coincidence between rhodopsin concentration and rod threshold demonstrable for only the final 10% of rhodopsin regeneration will coincide throughout the entire regeneration curve. The superposability of the regeneration curves for the final 10% of rhodopsin regeneration is additionally supported by an argument from the kinetics of this reaction. The regeneration of rhodopsin is estimated to follow the rate of a first order reaction indicating that the retinene supply is maintained relatively constant during the process. Such an assumption is especially justified during the final 10% of regeneration where the demands on retinene are least and the curve should be most accurately extrapolatable here.

The conclusion is reached that there is a fixed relation between scotopic thresholds and the fraction of opsin uncombined with retinene and the relation is mathematically defined by combining psychophysical functions on dark adaptation obtained from a study by Haig and those representing rhodopsin regeneration. The expression is log $I/I_D=ay/y_o$ and shows that the logarithm of threshold is directly related to the fraction of opsin (y) uncombined with retinene, and therefore rhodopsin concentration.

Significance to Program of Institute: The importance of relating the photochemistry of rhodopsin to rod threshold is considerable to the understanding of visual processes. The elegant mathematical expression demonstrating the logarithmic relationship between scotopic thresholds and rhodopsin concentration puts this relationship for the first time on firm quantitative grounds.

Proposed Course of Project: The project was completed during this calendar year.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Rushton, W.A.H., Fuortes, M.G.F , Gunkel, R D.: Increment Thresholds in a Subject Deficient in Cone Vision. J. of Physiol., (In Press), 1960.

Rushton, W.A H.: Dark Adaptation and the Regeneration of Rhodopsin, J. of Physiol. (In Press), 1960.

Rushton, W.A.H., Fuortes, M.G.F., Gunkel, R.D.: Rhodopsin Measurement and Dark Adaptation in a Subject Deficient in Cone Vision. J. of Physiol. (In Press), 1960.

Rushton, W.A.H.: The Intensity Factor in Vision. Symposium - Life and Light, The Johns Hopkins University Press. (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 40 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigations on Spectral Sensitivity of Diurnal Squirrels. (ERG Reactions of Pure Cone Mammalian Retinae)

Principal Investigators: Katherine Tansley, D.Sc.
Richard M. Copenhaver, M.D.

Other Investigators: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: .60
Professional: .60
Other: 0

Patient Days: None

Project Description:

Objectives: to study the spectral sensitivity curves of several species of squirrels in view of the possible existence of a second visual mechanism in this pure cone retina which is maximally sensitive to the shorter wave-lengths.

Methods Employed: Four species of ground squirrel and two species of tree squirrel were studied. Histological preparations were made from the eyes of all species.

Electroretinograms were recorded in response to a light stimulus provided by a Xenon arc lamp and double interference filters. Experiments were always conducted under both light and dark adapted conditions, and colored stimuli were tested throughout the whole spectral range Spectral sensitivity curves were plotted using the electroretinogram as the criterion.

In some cases the experimental eye was adapted to colored light which was on for five minutes before the experiment and during the run.

Major Findings: The retinae of all seven species examined showed the characteristics of a cone retina.

The spectral sensitivity curves were all found to be double humped, with maxima at about 535 and 490 mu. The humps were occasionally of the same height but more often one or the other was markedly higher. None of the experimental conditions was found to produce one type of curve rather than the other. Statistical analysis of the electrophysiological data resulted in a mean curve combining all the species examined. This curve was nearly symmetrical with the two humps of about the same magnitude. In addition there was a small but distinct reflexion between 450 and 460 mu.

Adapting the eye to color depressed the general sensitivity of the retina, but when results were calculated as percentage of the maximum it was found that blue adaptation depressed the hump at 490-500 mu while green or orange depressed the hump at 535 mu. The depression of the "blue" peak by blue light and the "green" peak by green light suggests the existence of two photosensitive mechanisms of different spectral sensitivities. Dominance of one peak over the other as found in the individual experiments suggest some sort of rivalry between the two mechanisms.

In view of recent work on photosensitive pigments (Dartnall 1960) it is postulated that the 535 mu peak is the result of interaction of some type between two photosensitive substances with absorption maxima at 502 and 480 mu and, that the 480 mu pigment is responsible for the peak in this region. If the two pigments are formed from one another as is believed, this would explain the apparent rivalry of the mechanisms responsible for the two peaks, since the more of one pigment present, the less of the other.

Significance to Program of Institute: Investigations on the pure cone retina of the squirrel is most helpful in providing information on the responses of a purely photopic mechanism uncontaminated by those of the scotopic mechanism. Such information may assist in the solution of a great difficulty encountered in studying the human electroretinogram, i.e. the separation of the cone response from the rod response.

Proposed Course of Project: Dr. Tansley has completed her study during 1960 during the time when she had terminated her appointment as visiting scientist of the Branch. Her plans on continuing this study are not known.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Tansley, K., Copenhaver, R.M. and Gunkel, R.D.: Some Aspects of the Electroretinographic Response of the American Red Squirrel, Tamiosciurus Hudsonicus Loquax. J. Cell. Comp Physiol. (In Press), 1960.

Tansley, K., Copenhaver, R.M., Gunkel, R.D.: Some Observations on the Off-Effect of the Mammalian Cone Electroretinogram. J. Opt. Soc. Am. (In Press), 1960.

Tansley, K., Copenhaver, R.M. and Gunkel, R.D.: Spectral Sensitivity Curves of Diurnal Squirrels. J. Vis. Res., (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 41 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Some Aspects of Electroretinographic Responses in the American Red Squirrel

Principal Investigators: Katherine Tansley, D.Sc.
Richard M. Copenhaver, M.D.
Ralph D. Gunkel, O.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1960):
Total: 1.60
Professional: .80
Other: .80

Patient Days: None

Project Description:

Objectives: To study the off-effect of the electroretinogram and the flicker fusion frequencies during dark adaptation in one of the members of the squirrel family, the American red squirrel.

Methods Employed: Placement of the electrodes, light stimulus and the general experimental details were only slightly modified as compared to the previously reported studies. The responses were amplified, displayed on an oscilloscope and photographed. For each adaptation curve the eyes were exposed for five minutes to the full stimulus of 7500 lux. Records were taken at intervals of ten seconds each for three minutes using a flash duration of 250 msec. The curves were analyzed particularly with respect to the off-effects. In the flicker experiments a sectored disc was used, the speed of which could be varied so that frequencies between 17 and 170 flashes per second were obtained. Critical flicker fusion frequencies were studied on

the second beam of the oscilloscope. After termination of the experiment the eyes were studied histologically following the in vivo fixation of the animal through the ascending aorta.

Major Findings: Some of the responses of the American red squirrel differ from the other species investigated. During dark adaptation the off-effect of the red squirrel electroretinogram decreases in amplitude, whereas, in other squirrel species it increases. Also, the flicker fusion frequencies of the red squirrel is considerably lower than in ground squirrel and chipmunk. Histological examination of the red squirrel retinae shows it to have a less complex inner nuclear layer and fewer ganglion cells. It is suggested that faster responses of the ground squirrel which result in a higher flicker fusion are due to a more efficient production of inhibitions and that this is associated with the remarkable development of the inner nuclear layer in this species. It is also suggested that the interaction of P-III and P-II may be more important for the development of the off-effect in the red squirrel while in the ground squirrel the action of P-II at "off" may play a big part.

Significance to Program of Institute: The red squirrel and most members of the squirrel family possess pure cone retinae. The responses obtained from such animals, then, is helpful in providing information on the responses of a purely photopic mechanism, not contaminated by those of scotopic mechanisms. A comparison of electrophysiological phenomena and the results of histologic examination provides some insight into the fundamental mechanism of off-effects.

Proposed Course of Project: Dr. Tansley has completed her study during 1960 during the time when she had terminated her appointment as visiting scientist of the Branch. Her plans on continuing this study are not known.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Tansley, K., Copenhaver, R.M. and Gunkel, R.D.: Some Aspects of the Electroretinographic Response of the American Red Squirrel, Tamiosciurus Hudsonicus Loquax. J. Cell Comp. Physiol. (In Press), 1960.

Tansley, K., Copenhaver, R.M. and Gunkel, R.D.: Some Observations on the Off-Effect of the Mammalian Cone Electroretinogram. J. Opt. Soc. Am. (In Press), 1960.

Tansley, K., Copenhaver, R.M. and Gunkel, R.D.: Spectral Sensitivity Curves of Diurnal Squirrels. J. Vis. Res. (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 42 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Some Observations on the Off-Effect of Mammalian Cone Electroretinogram

Principal Investigators: Katherine Tansley, D.Sc.
Richard M. Copenhaver, M D.
Ralph D. Gunkel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.60
Professional: .80
Other: .80

Project Description:

Objectives: The off-effect in the electroretinogram has not received attention in the past as have earlier parts of the response pattern. This is explained by the fact that in most species of laboratory animals, a good off-effect is missing. In the pure cone retina, on the other hand, a marked off-effect is a characteristic feature. In the present study, the squirrel was selected as the experimental animal.

Methods Employed: The experiments were carried out mainly on the ground squirrel Citellus beecheyi. The findings were confirmed in three other squirrel species all with pure cone retinae. The technical procedure was similar to that described in previous reports. Oscilloscope recording was used, and for flicker fusion tests arrangements were made to allow varying the total stimulus from 10 to 200

microseconds. Intensity response curves were taken for a-wave, b-wave and off-effect. The intensity of the white light of the Xenon arc was altered by means of neutral density filters. The rather deeply pigmented crystalline lens of the squirrel was extracted before recording was begun with the desired effect that the stimulus intensity at the retina was increased.

Major Findings: The intensity-response curves for the various parts of the squirrel electroretinogram showed that areas with increasingly high intensity stimuli, the a-wave and b-waves increased in amplitude, the off-effect was often depressed. During the development of the normal off-effect a second superimposed a-wave was much enhanced while a second superimposed b-wave was depressed. The a-wave returned to its normal value about 100 microseconds after off while the b-wave was slower, taking approximately 200 microseconds. When the off-effect was depressed by a strong stimulus, the enhancement of the second a-wave was much reduced and was somewhat delayed. The reactivation of the second b-wave was very much delayed. The results strongly support Granit's original supposition as to the nature of the off-effect in post excitatory inhibition. It was thought that the cone off-effect is not simply due to a quicker disappearance of P-III, leaving the remaining P-II to show itself as a new positive wave. There seems to be a reactivation of P-II at off--probably also a reactivation of P-III.

Significance to Program of Institute: Studies on the pure cone retina provide new information on the controversial issue of post excitatory inhibition and contributes greatly to basic problems of electroretinogram analysis.

Proposed Course of Project: Project is terminated.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Tansley, Katherine, Copenhaver, Richard M., and Gunkel, Ralph D.: Some aspects of the electroretinographic response of the American red squirrel, Tamiosciurus Hudsonicus Loquax. J. Cell. Comp. Physiol. (In Press), 1960.

Tansley, Katherine, Copenhaver, Richard M., and Gunkel, Ralph D.: Spectral sensitivity curves of diurnal squirrels. Journal of Vision Research. (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 43 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. Same as NINDB-88 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: A Study of the Proteins of the Lens

Principal Investigator: Robert A. Resnik, Ph.D.

Other Investigators: John Papaconstantinou, Ph.D.

Cooperating Units: Department of Microbiology
Johns Hopkins School of Medicine

Man Years (calendar year 1960): Patient Days: None
Total: 1.0
Professional: .5
Other: .5

Project Description:

Objectives: To obtain physical and chemical data on the lens protein.

Methods Employed: Methods and techniques commonly used in the physical-chemical characterization of polymers and polyelectrolytes, are used. In addition, techniques used for the studies of proteins are employed.

Major Findings: The transformation of alpha F and alpha G crystallins into particles of lower molecular weight is indicated by the appearance of slower sedimenting material in the ultracentrifuge patterns. The molecular weights of native and fully transformed alpha G crystallin determined by the approach to equilibrium method in the ultracentrifuge are 1,086,000 and 514,000 respectively. The sedimentation coefficient of a 0.29% solution of alpha G crystallin

in 0.05M phosphate buffer at pH 6.8 is 20.1 X 10^{-13}. The diffusion coefficient of this same sample is 2.0 X 10^{-7}. These values give a molecular weight of 940,000. A 0.41% solution of fully transformed alpha G crystallin gives sedimentation and diffusion values of 14.6 X 10^{-13} and 2.70 X 10^{-7} respectively. These parameters give a calculated molecular weight of 509,000. This pH induced decrease in the molecular weight of alpha G crystallin is irreversible and obviates Bon's discussion of a change in shape without a change in size. The net charge of the molecule at pH 8.7 does not appear to be altered since boundary electrophoresis does not reveal the two forms of alpha G crystallin when known mixtures were used.

Significance to Program of Institute: The identification of those proteins in the lens which are particularly susceptible to alteration or interaction brings us one step closer to a basis for correlating chemical changes in the lens with its transparency.

Proposed Course of Project: Changes in the C- or N-terminal amino acids and titration data may give some indication of the peptide bonds broken when alpha G crystallin is cleaved at alkaline pH. Further studies on the other alpha crystalline will be carried out to see if they react similarly at alkaline pH.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Resnik, R.A., Wanko, T. and Gavin, M.A.: Observations on a cytoplasmic component in lens fibers. J. Biophys. Biochem. Cytol. 7:403-406, 1960.

Papaconstantinou, J. and Resnik, R.A.: Biochemistry of bovine lens proteins I. Purification and characterization from the adult. J. of Biol. Chem. (Submitted for publication), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 44 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Structure of Ganglioside

Principal Investigator: Robert A. Resnik, Ph.D.

Other Investigators: Eberhard G. Trams, Ph.D.
Richard Irwin, Ph.D.

Cooperating Units: Section on Clinical Pharmacology
Section on Lipid Chemistry
NINDB

Man Years (Calendar year 1960):
Total: 1.0
Professional: .5
Other: .5

Patient Days: None

Project Description:

Objectives: To obtain and correlate physical, chemical and pharmacological data on ganglioside.

Methods Employed: The techniques of light scattering, conductivity measurements, and ultracentrifugation, in addition to other methods for the characterization of micelles will be used. General chemical methods for the determination of component parts of the molecule will also be employed. Standard pharmacological procedures for the assay and determination of the mechanism of physiological membrane phenomena are also utilized.

Major Findings: The molecular weight of brain ganglioside in water, determined by the Archibald procedures is around 300,000. In N, N-dimethylformamide the molecular weight is 3,000. This apparent dissociation is easily explained if this glycolipid is regarded as having properties similar to those of long chained organic molecules such as detergents. These form micelles above a certain critical concentration. The formation of these aggregates (micelles) is reversible so that by reducing the concentration below the "critical micelle concentration" the individual monomeric units exist in solution. This phenomenon of micelle formation is related to the solubility of the hydrophobic and hydrophilic portions of the molecule. An identical situation is found in ganglioside. Conductivity measurements bear out this analogy since in water there appears to be a critical concentration above which ganglioside exists as an apparent "macromolecule" and below which the true monomeric unit exists. The relation between this concentration (about 0.1%) and the concentration of ganglioside in membranes is not known.

Significance to Program of Institute: Considering the general role that ganglioside may have in muscle contraction and nerve conduction it is, aside from being chemically interesting, of value to the Institute program as a whole.

Proposed Course of Project: To determine the structure of ganglioside, to assess its role in membrane phenomena, and to study its chemical properties.

Part B included Yes ☐ No ☒

Serial No. NINDB-45 (c)
1. Ophthalmology Branch
2. Cell Biology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Rhodopsin Cycle in the Developing Mammalian Eye.

Principal Investigators: Sjoerd L. Bonting, Ph.D.
Leo L. Caravaggio, M.S.

Other Investigators: Peter Gouras, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2.0
Professional: .5
Other: 1.5

Patient Days: None

Project Description:

Objectives:

1) To establish the development of rhodopsin in the eye of the normal white rat and the DBA mouse from birth to maturity.

2) To establish the development and distribution of the enzymes retinene reductase and retinene isomerase in the eye of the same animals from birth to maturity.

3) To correlate the findings under 1) and 2) with the morphological development of the visual cell as studied by light microscopy and electron microscopy.

4) To correlate the findings under 1) and 2) with the functional development of the eye as studied by electroretinography (in collaboration with Dr. Peter Gouras.)

5) To establish the items under 1) through 4) for C3H mice and rats with hereditary blindness, and in rats treated with iodoacetate and glutamate.

Methods Employed: Rhodopsin is determined after dark-adapting the animals overnight. The animal is sacrificed, the eyes are removed and weighed, then the lens and vitreous are removed. The rest of the eye is treated for one hour in 4% alum, and is then homogenized in 1% digitonin (6 µl per mg eye) in a Potter-Elvehjem homogenizer. The homogenate is centrifuged at 10,000 x g for one minute and a 100-µl aliquot of the supernatant is transferred to a Lowry-Bessey microcuvette, containing 5-µl 1.0M NH_2OH. The extinctions at 500, 400 and 650 mµ are read in a Beckman DU spectrophotometer. All steps up till here have been carried out in dim red light. Then the solutions in the cuvettes are bleached for five minutes by a 100W light bulb at 8 cm. distance, and the extinctions at the same wave lengths are read. The rhodopsin concentration is expressed as follows:

$$\text{Rh. I.}_{500} = \frac{\Delta E_{500} \times \text{vol. (µl)}}{\text{weight (mg)}}$$

The absolute amount of rhodopsin per eye is calculated as follows:

$$\text{Rh. I.}_{\text{eye}} = \Delta E_{500} \times \text{vol. (µl)}$$

Major Findings: In the normal albino rat (Osborn-Mendel strain) rhodopsin begins to appear at 7d. The concentration (Rh. $I._{500}$) increases rapidly and practically linearly to 21 d., then becomes constant until maturity. The absolute amount of rhodopsin (Rh. $I._{eye}$) continues to increase, but after 21 d. at a slower rate.

The rhodopsin concentration (Rh. $I._{500}$) and the lengths of the whole rods as well as of the outer segments were plotted against age. From 0 to 14 d. there is a striking parallelism between the rhodopsin concentration and the outer segment length. The increase of the latter, however, becomes very slow after 14 d., at which age the rhodopsin concentration has reached only half of its maximum value.

The electroretinogram in these rats first appears at 10 d., after which the amplitude increases rapidly and almost linearly till 21 d. Beyond this age the amplitude remains about constant.

In C3H mice, which are blind through hereditary degeneration of the rods occurring between 10 and 16 d. after birth, a normal development of rhodopsin until 10 d. is found. Then the rhodopsin concentration begins to decrease and reaches virtually zero between 16 and 19 d.

Intravenous injection of 3 x 20 mg/kg iodoacetate over a 30-hour period in 8-week-old rats results, after 24 hours, in a 40% decrease of the rhodopsin concentration, without morphological changes visible in the light microscope.

Preliminary studies of the enzyme retinene reductase have been made with retina from the rat and the cow. The histochemical staining technique, with tetrazolium, has been found to be unsuitable because if indicates "diaphorase" activity rather than retinene reductase activity. Chemical assays in retinal homogenates, using ethylalcohol or acetaldehyde as substrates and following either DPN reduction or oxidation spectrophotometrically, were unsuccessful thus far because of low activities and the occurrence of non-specific and even of non-enzymatic oxidation-reductions.

Significance to Program of Institute: The primary interest of this study is in the correlation of biochemical, morphological and functional events in the developing visual receptor cell. The chemical findings of rhodopsin development in the normal rat eye correlate very well with recent studies by De Robertis and others on the morphogenesis of the rod ultrastructure. Rhodopsin begins to appear at the same time as the "rod sacs" in the outer segments, and parallels their further development until 14 d. At this time, the outer segment is completely developed while the rhodopsin concentration continues to increase till 21 d. Wolken recently calculated that in the mature eye the surface area of the rod sacs is sufficient to be covered entirely with a monomolecular layer of rhodopsin molecules.

Our results would indicate that between 14 and 21 d. the rod sac surface is not yet completely covered with rhodopsin molecules, or that rods are still developing in the peripheral areas of the retina (the determinations of the outer segment length refer to the central area of the retina, while the chemical determinations of the rhodopsin concentration involve the entire retina.) This point will have to be further clarified.

The findings in the C3H mice seem to rule out one possible explanation of the cause of their blindness. Since rhodopsin is formed in their eyes between 7 and 10 d., and then begins to decline at the same time as morphological degeneration of the rods starts, it is unlikely that the morphological events leading to blindness can be explained as due to an inborn failure to form rhodopsin.

The parallelism between the rhodopsin concentration and the electroretinogram is another important finding. This is in good agreement with recent work by Dowling and Wald on vitamin A-deficient rats.

Proposed Course of Project: Further integrated studies of the time course of rhodopsin concentration, rod morphology and electroretinogram will be undertaken in rats with hereditary retinitis pigmentosa, and in rats treated with iodoacetate and glutamate.

Attempts to work out an ultramicro assay procedure for retinene reductase will be continued. If successful, a study of its distribution in the retina and its development will be made with the quantitative histochemical technique.

Radioautographic studies with tritum-and C^{14}-labeled vitamin A alcohol and vitamin A acid are planned in the hope of further elucidation of the site and the dynamic aspects of rhodopsin formation.

It is hoped to prepare a ferritin-labeled antibody to rat opsin and to study its localization by electron microscopy to verify the assumption that rhodopsin is deposited on the rod sac membrane.

Part B Included Yes /___/ No. /_X_/

Serial No. NINDB-46 (c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Membrane ATP-ase and Aqueous Formation

Principal Investigators: Sjoerd L. Bonting, Ph.D.
Kenneth A. Simon, M.S., M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: 2.0
Professional: .5
Other: 1.5

Project Description:

Objectives:

1) In 1957 Skou described an ATP-ase in crab nerve which requires Na^+ and K^+ for its activity, is stimulated by Mg^{++} and inhibited by Ca^{++} as well as by very low concentrations of ouabain. More recently it was shown by Post and by Hoffman that a similar enzyme occurs in red blood cell ghosts and is closely connected with Na^+ and K^+ transport across the membrane.

2) It was decided to study the distribution of this enzyme in the mammalian organism, and to investigate in particular its possible relationship with aqueous formation and spinal fluid formation.

Methods Employed: The activity of the membrane ATP-ase is determined as the fraction of total ATP-ase activity which can be inhibited by omission of K^+ from the assay medium or by addition of 10^{-4}M ouabain to the medium. The tissue is frozen in liquid nitrogen immediately upon removal from the anesthetized animal. It is then weighed and a 10% (w/v) aqueous homogenate is prepared in a Potter-Elvehjem homogenizer. Aliquots of 0.5 ml. of the homogenate are frozen-dried in 100 x 13 mm test tubes and stored in the freezer until use. Immediately prior to use the homogenate is reconstituted with H_2O. From 5-30 µl of homogenate is mixed with 150 µl each of one of the following media:

a. 2 mM/l ATP (Na-salt), 45 mM/l NaCl, 10 mM/l NaCN, 5 mM/l KCl, 1 mM/l $MgCl_2$ and 0.1 mM/l versene in 0.1M TRIS buffer (pH 7.5).
b. medium a without KCl.
c. medium a with 0.1 mM/l ouabain added.
d. medium a with 1 mM/l Ca Cl_2 added.

Of each mixture 12 µl aliquots are incubated for 1 hour at 37°C, while non-incubated aliquots of the mixture with medium a are included as enzyme blanks. After adding 50 µl 10% trichloroacetic acid, the tubes are centrifuged, and 30 µl supernatant from each is transferred to tubes, containing 30 µl of a freshly prepared solution of 40 mg/ml $FeSO_4$ in 1.0% NH_4 molybdate in 1.15 N H_2SO_4. The optical density at 700 mµ, representing inorganic phosphate, is read in Bessey-Lowry microcuvettes in a Beckman model DU spectrophotometer.

The effects of other possible inhibitors on this enzyme are studied by using the same procedure, and substituting the compound studied for ouabain in medium c.

Major Findings:

1) Membrane ATP-ase activity was demonstrated in the following tissues of the cat: white matter, grey matter, optic nerve, choroid plexus, ciliary body, kidney cortex, medulla and papilla. In these tissues omission of K^+, addition of 10^{-4}M ouabain, or addition of 10^{-3}M Ca^{++} caused about the same relative inhibition.

2) No appreciable activity was found in: smooth muscle (iris, gut), striated muscle, heart muscle, liver, lung, and stomach mucosa.

3) The ouabain concentration at which 50% inhibition occurs in ciliary body was found to be 5 x 10^{-7}M, which is close to that reported by Post for erythrocyte membrane ATP-ase (10^{-7}M).

4) Diamox in 10^{-4}M concentration fully inhibited the membrane ATP-ase of ciliary body and choroid plexus.

Significance to Program of Institute: The demonstration of membrane ATP-ase activity in ciliary body and other secretory organs, and the unexpected finding of its inhibition by Diamox strongly suggest that this newly discovered enzyme may play an important role in aqueous formation. It is also further evidence in favor of the theory of active secretion in aqueous formation.

Proposed Course of Project:

1) The half-inhibition concentration for Diamox will be studied in ciliary body and choroid plexus.

2) The in vitro effect of other carbonic anhydrase inhibitors (Daranide, Neptazane, Cardrase) and of CL 17,262 (Lederle), an analogue of Neptazane which does not inhibit carbonic anhydrase, will be determined for the membrane ATP-ase of ciliary body and choroid plexus.

3) The effect of ouabain on the aqueous flow in normal persons and patients with glaucoma will be studied by means of tonography.

4) A study will be made of the effect of intravenous injection of ouabain and Diamox on the membrane ATP-ase activity in the choroid plexus and ciliary body of the cat.

5) Experiments with Na^{24} will be done in an effort to find a direct effect of ouabain on Na^{+} transport in the formation of aqueous fluid and spinal fluid.

Part B included Yes ☐ No ☒

Serial No. NINDB - 47 (c)

1. Ophthalmology Branch
2. Cytology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Influence of Alpha Chymotrypsin on the Healing of Corneal Endothelial Wounds.

Principal Investigator: Hubert Hückel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: Irrigation of the posterior chamber with alpha chymotrypsin is now widely used for intracapsular extraction of cataractous lenses in young and middle-aged patients. During the surgical procedure, injury to the endothelium of the cornea occufs frequently. The present investigation centers around the problem of interference of endothelial wound healing by the enzyme.

Methods Employed: The endothelial layer of both eyes of eight-to-twelve week old rabbits was injured with a spatula. Attempts were made to standardize the lesions. A chymotrypsin solution of 150 armour units per ml. was perfused into the anterior chamber and washed out with a 0.9% sodium chloride solution after three minutes. A mock procedure was performed on the second eye which served as control. At various time intervals, whole mounts of endothelium were prepared and studied microscopically for cell proliferation and mitotic activity. The colchicine technique was used to arrive at a more conclusive picture of mitotic activity in the injured eye. The effect of locally administered l-epinephrine on cell division was investigated in an additional series of experiments.

Major Findings: With the technique used, the produced lesions were not reliably standardized as to size and depths of the injury. Many experiments were necessary, therefore, to arrive at definite conclusions. When the animals were killed 24 hours after injury and treatment, the number of dividing cells were slightly less than in the control eye. This difference disappeared at a 48 hour interval. Division occurred predominantly in cells which surrounded the wound. In the corneal endothelium, colchicine treatment (1 mg./kg.) did not arrest endothelial mitosis in metaphase. Local instillation of l-epinephrine depressed mitotic activity and influenced also to some extent the counts of the other eye. It was concluded that in young, healthy rabbits, the depressant effect of chymotrypsin on cell division was of low order and transient, and that the use of the enzyme in cataract surgery cannot be considered as a hazard by delaying endothelial regeneration unduly.

Significance to the Institute: Certain undesirable side effects in the use of chymotrypsin for cataract surgery have been reported. Previous experimental data on uninjured cornea indicated that the endothelium tolerates the enzyme in the anterior chamber for several minutes. In the present study, it was brought out that wound healing in the endothelium is only slightly depressed by enzyme treatment.

Proposed Course of Project: It is planned to extend the experiment to older animals which might allow a closer comparison between the experimental conditions and the human cataract surgery.

Part B Included Yes /___/ No. /_X_/

Serial No. NINDB - 48 (c)
1. Ophthalmology Branch
2. Electron Microscopy Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Electron Microscope Study on the Protozoon *Toxoplasma gondii*

Principal Investigator: Theodor Wanko, M.D.

Other Investigators: Leon Jacobs
Jack Remington

Cooperating Units: Laboratory of Parasitic Diseases, NIAID

Man Years (calendar year 1960): Patient Days: None
Total: .40
Professional: .20
Other: .20

Project Description:

Objectives: To determine by means of the electron microscope the formation and fine structure of Toxoplasma cysts and to compare the encysted organisms with those obtained from peritoneal exudate.

Methods Employed: Initally, Toxoplasma cysts were collected by a micromanipulatory procedure from homogenized, infected mouse brain. The sampled cysts were then sedimented and the residue thus obtained was fixed, dehydrated and embedded for sectioning.

Subsequently, blocks from the cerebral cortex of white, weanling mice of the NIH general purpose strain previously infected by subcutaneous innoculation at 17, 60 and 200 days, were fixed, dehydrated and embedded. The inoculum consisted of 0.25 ml. of a 1:10 dilution of mouse brain homogenate containing cysts of the Beverly strain of Toxoplasma.

Comparative material of Toxoplasma organisms was obtained by fixing and centrifuging peritoneal exudate of acutely infected mice. The sediment was then dehydrated and embedded.

The embedding media were methacrylate and araldite and the microtome employed was a Servall model. An RCA electron microscope model EMU 3C is used. Control and comparative examinations are carried out with the aid of phase contrast and light microscopy.

Major Findings: The sedimented, isolated cysts studied in the initial phase of this project provided some information as to the presence and gross arrangement of organisms inside the cyst wall. For the following reasons this method of investigation was abandoned:

1. The isolation of cysts from their natural milieu prevents the simultaneous observation of the surrounding tissue.

2. In the micromanipulatory sampling only the largest cysts are selected and therefore little information on cyst development can be expected.

3. The cyst material, though biologically still active, was not adequately preserved, presumably because of the time delay necessary for homogenization and isolation.

The Toxoplasma cysts examined in sections of brain tissue blocks show that the organisms are encapsulated by an opaque layer wherein vesicular and membranous elements can be resolved. Within the cyst, the space between the cell boundaries of the individual organisms is filled with a homogeneous material which is in continuity with the capsule and is of similar opacity. At present, the observations may indicate that the opaque material between the protozoa and in the cyst wall derives from the organisms whereas the membranous and vesicular profiles in the cyst wall may correspond to remnants of host cell material. Individual organisms have been seen in the lumen of brain capillaries and also intracellularly adjoining the outer surface of the capillary without being encysted.

The cellular organization of the organisms in the cyst is characterized by the presence of the following constituents: nucleus with nucleolus; mitochondria; Golgi complex; endoplasmic reticulum; toxonemata, emanating from the conoid; a sub-membranous, fibrillar apparatus; vacuoles; and a continuous cell membrane.

The encysted protozoa closely resemble those seen both intra- and extracellularly in mouse peritoneal exudate. The only apparent difference is seen in the presence of a number of vacuoles with a homogenous content of low opacity in the encysted organisms.

Significance to Program of Institute: This study may serve to provide information on the mechanism involved in the formation of the Toxoplasma cyst as it occurs in uvea and brain in human Toxoplasmosis. Knowledge of the composition of the cyst capsule may lead to a method to counteract the encysted organisms which heretofore have been resistent to therapy.

Proposed Course of Project: Examinations of infected brain will be continued for the purpose of obtaining more morphological data on progression of the organism from the lumen of the blood vessel into the brain and the early phases of cyst formation.

Part B included Yes [ ] No [X]

Serial No. NINDB - 49 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB 103 (c)

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Electron Microscope Studies on Biopsies of Human Muscle Disease

Principal Investigators: Theodor Wanko, M.D.
G. Milton Shy, M.D.

Other Investigators: Mary Ann Gavin, M.S.

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: .80
Professional: .40
Other: .40

Project Description:

Objectives: To determine by means of the electron microscope detectable change in human muscle affected with various dystrophic diseases as compared with normal tissue.

Methods Employed: Material obtained from muscle biopsies on human subjects with muscle dystrophies of various kinds are immersed immediately after excision in 1% osmium tetroxide for fixation. This is followed by dehydration and, in some cases, by additional impregnation with 1% phosphotungatic acid, embedding in methacrylate and araldite and sectioning on a Servall and an LKB ultra-microtome.

An RCA electron microscope, Model EMU-3C, is used in these studies. Control and comparative examinations are carried out with the aid of phase contrast and light microscopy.

Major Findings: During the last year, two diseases, Familial Periodic Paralysis and Central Core Disease were singled out for systematic study.

I. Familial Periodic Paralysis: In one case of familial periodic paralysis, biopsy specimens from both the phase of paralysis and of remission were analyzed. The pathological changes found in these instances concern the sarcoplasmic reticulum, mitochondria and and the sarcoplasmic matrix. The cysternal spaces of the sarcoplasmic reticulum appear enlarged to various degrees and thus distort the regular arrangement of myofibrils in the cell. The slightly enlarged cavities of the reticulum usually show no content whereas the greatly enlarged portions frequently are filled with granular material of low opacity which has an average diameter of 250 Å. The granules are observed also outside these loculated areas in the cytoplasmic matrix usually in a rather scattered distribution. Circumstantial evidence relates their identity with a glycogen protein complex.

The mitochondrial changes consist of increase in size and transformation from a rodlet to a rounded corpuscle. At the same time, the mitochondrial matrix between the cristae may appear light and swollen. Another form of mitochondrial change is observed mostly in the area beneath the sarcolemma. There normal mitochondrial profiles are in close contact or are in continuity with structures outlined by a single contour which contains circular or irregular profiles that are somewhat indistinct and of low opacity. These altered mitochondria frequently are studded with very small and extremely opaque granules.

The described pathological formations are found in the muscle taken during paralysis, as well as in that from a period of remission. Their extent, however, varies greatly with the clinical state in so far as in the paralytic phase the changes in sarcoplasmic reticulum are abundant whereas during remission they are sparse.

II. Central Core Disease: The fine structural pathology of central core disease is currently under study. The biopsy specimen was taken from one member of the family where this clinico-pathological syndrome was first reported (Shy and Magee, 1956). The changes in fine structure found in this case corroborate certain light microscopic findings. The transition from the normal to the core area is rather abrupt. The predominant structural elements in the central core area are filaments which morphologically are indistinguishable from their counterparts in the cell periphery. Their grouping into fibrillar units, however, is either ill-defined or completely absent. As a rule, no interfibrillar space nor mitochondria can be discerned. Occasionally, vesicular profiles which may be part of the sarcoplasmic reticulum are dispersed in this region. The pattern of muscle cross banding in the central core differs from that seen peripherally.

First, the length of the individual sarcomeres appears reduced and the Z lines are out of phase as compared with the adjacent normal fibrils. Secondly, the width of the I bands may gradually be lessened to such a degree towards the center of the core that in some cases it is not discernable at all. Thirdly, the Z bands assume a zig-zag appearance and in the center of the core they sometimes grossly deviate from their plane which normally would be perpendicular to the fibrillar axis. Finally, at the innermost portions of the core the parallel, longitudinal alignment of the myofilaments is changed to an irregular and tortuous distribution. Here, thicker filamentous elements can be observed intermingled with normal myofilaments; they are of indefinite length and pursue a tortuous course. Their opacity corresponds to that regularly seen in the Z disc region.

Significance to Program of Institute: The data obtained from the electron microscope study on Familial Periodic Paralysis were integrated with results obtained in biochemical, metabolic, physiological, histological, histochemical and clinical investigations. The concurrence of data from these various disciplines provided a basis for the theory which interprets this disease as a disturbance in carbohydrate metabolism.

The data obtained from the electron microscope investigation of Central Core disease indicate the absence of an essential cytoplasmic element, namely mitochondria, in the core. There also may occur a change in the quantitative distribution of components which are part of the contractile elements.

Proposed Course of Project: Studies on normal muscle will be continued. Specimens of various kinds of neuromuscular diseases are continuously collected. The results obtained on Familial Periodic Paralysis may be supplemented by data obtained from cases of the same or similar diseases.

The results of the investigation of central core disease will be compared with findings obtained from histological and histochemical methods.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Shy, G.M., Wanko, T., Rowley, P.T., and Engel, A.G.: Studies in Familial Periodic Paralysis. Experimental Neurology (In Press), 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 50 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-104 (C)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Electron Microscopic Studies on Tissues of the Eye, such as Epithelium, Fibers and Capsule of the Lens, Epithelium of the Ciliary Body, the Optic Nerve and the Conjunctiva

Principal Investigators: Theodor Wanko, M. D.

Other Investigators: Mary Ann Gavin, M. S. and Ludwig von Sallmann, M. D.

Cooperating Units: None

Man Years (Calendar Year 1960)
Total: .80
Professional: .40
Other: .40

Patient Days: 190
Conjunctiva: 753

Project Description:

Objectives: 1) To investigate the normal characteristics of lens tissue elements as seen in the electron microscope, 2) to investigate the ultrastructure of the lens epithelium and the lens fibers after cataractogenic agents had been administered, 3) to examine human cataractous lenses, obtained immediately after operation, in order to gain insight into the submicroscopic cellular pathology, 4) to study the morphology of the ciliary epithelium with the electron microscope. Information on the ultrastructure of the epithelium may lead to an understanding of its function, and particularly, its role in the formation of aqueous humor, 5) to study the normal characteristics of human conjunctival ultrastructure which should serve as a control in examination of pathological tissue.

Methods Employed: An RCA electron microscope, Model EMU-3C, is used in these studies. Control and comparative examinations are carried out with the aid of phase contrast and light microscopy. The tissues are obtained either from the anesthetized animal and immediately immersed in the fixation medium, or fixation is started in situ. Biopsy specimens of conjunctiva and human cataractous lenses are immersed in the fixative immediately following excision. All tissues are then dehydrated in alcohol, dissected, and embedded in methacrylate as well as araldite. The addition of 0.075 per cent uranyl nitrate to the embedding mixture proved to result in better preservation of tissues during polymerization and also serves to enhance contrast. Sections are cut on Servall and LKB ultra-microtomes and transferred to collodion filmed grids covered with a fine carbon layer.

Major Findings:

1. Normal lens

The studies on the normal lens were continued as indicated in last year's report by the electron microscopic examination of the suture areas in the cortex of the normal mature rat and rabbit lens.

In the vicinity of the suture, the regular array of the lens fiber membranes gradually changes to a system of many protruding cell portions interposed between similar projections of the adjacent cells. These interdigitations occur in all directions between neighboring cells as well as those located in opposite lens portions. In this area of the lens, solely, specialized contact areas of cell boundaries, classified as desmosomes, are present.

From these data, together with previous observations on epithelium and fibers, it is apparent that the intercellular relationship which is characterized by numerous interdigitations predominates in those areas of the lens which undergo the greatest dimensional changes during accommodation. Thus these localized differentiations of the cell surface may preserve the morphological integrity of the cell during the process of accommodation.

2. Experimental Cataract

Myleran Cataracts (2, 4 and 6 weeks): No new data could be obtained from the material available and the observations have, therefore, been discontinued for the present.

Mimosine Cataracts (5, 7 and 14 days): The electron microscope observations on mimosine induced cataracts have been concluded. The data obtained are being considered for future publication.

3. Human Cataract

At present, eight lenses with senile cataracts have been obtained. Last year, a lipofuscein type pigment was described in the epithelial

cells, the pathognomonic significance of which still remains doubtful. Other structural changes which discriminate the cataractous lenses from those of mature experimental animals involve the lens capsule, epithelium and cortical lens fibers. The normally homogenous lens capsule occasionally exhibits in the senile cataracts an interposition of dense granular material in a regular arrangement. In epithelium, as well as in cortical lens fibers, structures are observed which resemble fragmented or disintegrated cellular constituents. There is a considerable variation in character and extent of these abnormal formations within one lens, as well as, among the lenses investigated. It is felt, at the present time, that a large number of specimens from many lenses will have to be analyzed in order to determine common pathognomonic signs in comparison with normal human material.

4. Ciliary Body and Optic Nerve

Both projects were not continued during this past year.

5. Conjunctiva

One normal and nine pathological (Sjögren's syndrome) biopsy specimens have been collected. The investigation of these specimens is in progress. No conclusions can be reached as yet. It will be attempted to obtain more normal control material.

Significance to Program of Institute: The previous studies on normal and experimental, cataractous lenses provide a basis for the studies on the pathology of senile cataracts. The further collection of data might lead to information on fine structural changes and eventually their time sequence in senile cataracts. A similar goal is sought in the study of the fine structural organization and pathology of the conjunctival tissue.

Proposed Course of Project: Observations on both human cataracts and human conjunctival tissue will be pursued. More data and material on both projects will be assembled.

Part B included: Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Wanko, T. and Gavin, M.A.: Cell Surfaces of the crystalline lens. In "Symposium on Structure of the Eye" (G. Smelser, Ed.) Academic Press, New York (In Press).

Wanko, T.: "The Crystalline Lens. Electron Microscopic Investigation of the normal fine structure and of experimentally induced cataracts." Exhibit at the 28th Annual Scientific Assembly of the District of Columbia. November 1959, Washington, D. C.

Wanko, T.: "The Crystalline Lens. Electron Microscopic Investigation of the normal fine structure and of experimentally induced cataracts." Exhibit at the Annual Meeting of the Aero Space Medical Association, Miami, Florida, May 1960.

Honors and Awards:

Certificate of Merit by the Medical Society of the District of Columbia for exhibit on the Crystalline Lens, November 1959, Washington, D. C.

Serial No. NINDB - 51 (c)
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. Same as NINDB 86 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The role of the vasculature in the maintenance of intraocular pressure

Principal Investigator: Frank J. Macri, Ph. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: .90
Professional: .40
Other: .50

Patient Days: None

Project Description:

Objectives: To determine if and by what mechanisms the vasculature of the eye may effect the intraocular pressure.

Methods Employed: Intraocular pressure (IOP) and vascular pressures in various parts of the eye and body were determined. Correlations were made between the IOP and the vascular pressures to show the relative dependence or independence of the various systems.

Major Findings: 1) Interrelationship of intraocular pressure and venous pressure (cat) - Simultaneous measurement of pressures in the anterior ciliary vein and vortex vein have been found to be identical. This is explainable by the fact that these two vessels are in direct communication by numerous intrascleral anastomoses as shown by the preparation of casts.

In a series of 39 cats, simultaneous measurements of "normal" intraocular pressures and "head-on" venous pressure were made at a time when two variables had been constant for at least fifteen minutes. The IOP when plotted as a function of the VP produced a linear regression which was highly significant ($P < .001$) and could be expressed by the equation IOP = 0.82 VP + 6.51. In these experiments, the method of measuring VP necessitated the total occlusion of the vein and cast some doubt on the validity of the findings. The relationship was, therefore, reinvestigated by the use of microcannulae inserted through the wall of the vein, upstream so that the vessel remained patent. From nine such experiments, a very highly significant ($P < .001$) regression was obtained which was almost identical to that obtained previously and could be expressed by the equation IOP = 0.83 VP + 6.51. Since the total occlusion of one vein in the first experiments had no obvious effect on the IOP/VP relationship but produced a mean rise of 20 mm. Hg. in the IOP, it appeared that the venous pressure may be the dominant factor in the maintenance of the IOP. The validity of this concept was, therefore, studied in another series of experiments.

A. Intact eyes of anesthetized cats. Venous pressure measured head-on. Acute changes of blood pressure were produced by the administration of various agents which in turn produced acute changes in the IOP and VP. The induced changes of the two pressures were plotted against each other so that an IOP/VP relationship could be obtained for individual eyes over a fairly wide range. The curves of 12 such experiments (cats) were grouped and could be expressed by the equation IOP = 0.90 VP + 3.42. No significant difference was found between this group and the previously mentioned 39 cat series.

B. Enucleated, arterially perfused eyes. (1) A series of 45 eyes were enucleated and perfused through the ophthalmic artery with Eagle's Basal Medium (EBM) containing 10% cat plasma. Single determination of intraocular pressure and "head-on" venous pressure of each eye at steady state produced a highly significant linear regression ($P < .001$) which could be expressed by the equation IOP = 0.77 VP + 7.43. This regression was not significantly different from the 39 cat series.

(2) A series of eleven eyes were perfused as above but the IOP and VP were varied in each eye by raising and lowering the perfusion pressure. The relationship of IOP/VP was, therefore, made for the individual eyes and the slopes and intercepts of each eye grouped. The mean equation obtained was IOP = 0.89 VP + 3.58, again not significantly different from the 39 cat series.

It is, therefore, apparent that the different procedures used to produce a primary effect on the vasculature of the eye all produced a relationship of IOP/VP similar to that found in "normal" eyes.

Since excellent correlations were obtained between IOP and VP when VP was varied, in both intact and enucleated eyes, it did not appear probable that varying the aqueous humor volume would produce similar correlations. This was tested, however, in eight eyes. The aqueous

volume was increased then decreased by increments, so that simultaneous measurements of VP and IOP could be made. It was found when the IOP was raised above opening pressures, that the VP fell; lowering of the IOP by decreasing the volume also caused a fall of VP. The latter fall of VP was found to be correlated with the IOP so that a mean curve could be expressed by the equation IOP = 1.74 VP - 18.87. This equation is very significantly ($P < .001$) different from those obtained in the 39 cat series.

In summary, it may be stated that since changes of VP produced values of "K" in the relationship IOP = K VP + C, similar to that obtained in "normal" eyes and varying the volume of aqueous humor resulted in a significantly different "K", it must be concluded that the IOP of the eye is dependent in a large part on the venous pressure.

2) Vascular pressure relationships and the intraocular pressure - The intraocular pressure of the eye had been previously reported to be related to the anterior ciliary or vortex venous pressure; however, no relationship has been found between the normal eye pressure and systemic blood pressure. On the other hand, it is well known that acute changes of blood pressure produce parallel changes of eye pressure. It would appear, therefore, that some slowly compensating mechanisms exist to explain the lack of correlation of intraocular pressure (IOP) and systemic blood pressure (B.P.). The two most likely possibilities for this are alterations of aqueous humor dynamics (outflow and inflow) and/or vascular pressure dissociations occurring somewhere between the heart and the exit veins of the eye. The latter possibility, of vascular dynamics, was explored.

To determine pressure correlations between adjacent arterial systems, measurements were made to correlate (1) systemic blood pressure and ophthalmic artery pressure, (2) ophthalmic artery and iris artery pressures, and (3) iris artery and intraocular pressures. It was thought that if these blood vessels were not under direct and active physiologic control, good correlations should be obtained for the various pairs. A dissociation of pressure within any of the paris, however, might indicate "control" to one vessel of the pair. Such observations, therefore, might shed more light on the overall role of the vasculature in the maintenance of intraocular pressure.

It must be stressed that the methods employed to measure iris artery and ophthalmic artery pressures interfere with blood flow. Insertion of the cannulae into the lateral long posterior ciliary artery to measure iris artery pressure necessitates total occlusion of this vessel. However, if the intraocular pressure is influenced appreciably by the iris artery pressure over a relatively wide range , then under this condition no untoward effects of the procedure should be notable on the relationship of IOP to the IAP. Pressure of the femoral artery was used as representative of the systemic blood pressure, due to its easy accessibility with the experimental setup used.

A very high correlation was found between the femoral artery and ophthalmic artery pressures; so that it must be concluded that the ophthalmic artery pressure varies continuously with the general blood pressure of the animal. Significantly, no correlation could be found between the pressures of the ophthalmic artery and the iris artery under the conditions of the experiment whereas a highly significant correlation was obtained between the iris artery and the intraocular pressure.

It is concluded from the data presented that the pressure in the iris artery is not associated with the general blood pressure and that a dissociation occurs at the level between the iris artery and the ophthalmic artery. The mechanism by which the dissociation is brought about is not known.

It has been shown that a relationship exists between the IOP and the venous pressure which can be expressed by the equation IOP = 0.82 VP + 5.46. The equation found in these experiments which related IOP to the iris artery was IOP = 0.82 IAP - 4.90. Since the slopes of both equations are the same, it becomes apparent that a 1:1 relationship exists between the venous pressure and the iris artery pressure.

Significance to Program of Institute: It was the concensus of opinions in the past that the IOP was maintained by changes in volume of the aqueous humor.

In the anesthetized cat, as well as in arterially perfused enucleated cat eyes, it was found that the IOP is mainly supported by the vasculature of the eye, primarily at the level of the iris arteries. The significance of these findings for the human is not known, but certainly does suggest that similar studies be carried out on eyes of higher species.

Proposed Course of Project: It is planned to repeat these experiments in arterially perfused enucleated rabbit and monkey eyes as well as in the intact eyes of these two species.

Part B included: Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Macri, F. J.: Vascular Pressure Relationship and the Intraocular Pressure, A.M.A. Arch. Ophth. (in press).

Macri, F. J.: A Study of the Interdependence of the Intraocular Venous and Eye Pressure, A.M.A. Arch. Ophth. (in press).

Honors and Awards relating to this project: None

Serial No. NINDB - 52 (c)
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. Same as NINDB-87 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study on the Pharmacodynamics of Various Agents Affecting Intraocular Pressure

Principal Investigator: Frank J. Macri, Ph. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: .90
Professional: .40
Other: .50

Patient Days: None

Project Description:

Objectives: To determine the pharmacodynamics of agents effective in altering the intraocular pressure with a view toward finding more effective agents and perhaps elucidating more clearly the mechanisms which maintain the intraocular pressure.

Methods Employed: In another report it was noted that the slopes of the relationships IOP/VP and IOP/IAP were both found to be 0.82 in untreated eyes. In subsequent experiments it was found that epinephrine and arterenol, administered intravenously, did not alter the IOP/VP relationship, but in a large number of cases the slope of the IOP/IAP relationship was markedly reduced, i.e. the iris artery pressure fell much more in relation to the IOP than could be predicted by the slope previously determined. It was felt that such an effect could be obtained if the iris artery had been constricted by these agents.

In order to test this hypothesis, it was deemed necessary to measure the iris artery pressure in an isolated perfused iris in order to avoid complications from other systems of the eye. Also necessary was a measurement of the rate of flow of perfusate to indicate changes of resistance of the blood vessels which would in turn indicate calibre changes. A small differential-pressure type flowmeter was constructed which could demonstrate changes of flow in the order of 8 cmm/min. The iris was removed from the enucleated eye with the two long posterior ciliary arteries (LPCA) intact. The perfusate system, with the flowmeter in line, was connected to one of the LPCA's by means of a fine polyethylene cannula. A second cannula was inserted into the other LPCA for the measurement of iris artery pressure. A number of perfusate bottles all maintained under the same pressure were connected to the main line of the system. Thus, by a turn of a stopcock, flow could be switched from one perfusate bottle to another.

Major Findings: 1) Action of epinephrine and arterenol - Epinephrine and arterenol in concentrations of 10^{-12} produced a rise of iris artery pressure and a decrease of perfusate flow. These agents in doses of 10^{-6} produced only a fall of IA pressure with decreased flow. Doses, in between the latter two, generally produced biphasic responses beginning first with a rise of IA pressure which then proceeded to fall below the control levels. All these responses were reversible upon reinstitution of the control perfusate.

The mechanism of action proposed for these agents is:

a. Low doses - produce a constriction of the radial arteries of the iris.
b. High doses - produce a constriction also of the iris artery.

2) Action of acetylcholine (Ach.) - Only the action of low doses of Ach have been completed at this time. Perfusate containing eserine were used. Ach (10^{-12}) was found to lower the iris artery pressure and to increase perfusate flow. This action is explainable on a basis of dilatation of the radial arteries of the iris.

3) Action of acetazolamide (Diamox) - As above, only the effects of low doses of Diamox have been determined to date. Doses of 5 mg/l (approximately equal to the human dose) produces a rise of iris artery pressure and a decrease of perfusate flow. This effect is compatible with a constriction of the radial arteries of the iris.

Significance to Program of Institute: The fact that the agents studied have an effect on the vasculature of the eye throws a light on the physiology of the intraocular pressure and on the pharmacodynamics of the drugs which affect it. It was previously felt that the carbonic anhydrase inhibitors and sympathomimetic amines reduced IOP by diminishing the secretion of aqueous humor. In the eyes studied (cat), at least part of the lowering of IOP induced by these agents can be ascribed to a constriction of the iris arteries. The role of the iris artery

pressure is thus brought to the forefront for investigation of IOP regulatory mechanisms.

Proposed Course of Project: This project will be continued with emphasis on the action of anti-cholinesterase compounds.

Part B included: Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

PART B Honors, Awards, and Publications

Publications other than abstracts from this project:

Macri, Frank J.: Acetazolamide and the Venous Pressure of the Eye. A. M. A. Arch. Ophth. 63:953-965, 1960.

Macri, Frank J.: Further Studies on the Action of Acetazolamide and the Venous Pressure of the Eye. A. M. A. Arch. Ophth. 64:550-552, 1960.

Honors and Awards relating to this project: None

Serial No. NINDB - 53 (c)
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Observation of the Flow Pattern in the Ciliary Processes of Albino Rabbits. An in vitro study.

Principal Investigator: Luigi Scullica, M.D.

Other Investigators: Frank J. Macri, Ph.D.

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: 1.2
Professional: 1.2
Other: 0

Project Description:

Objectives: The vascular network of the ciliary processes was previously studied by means of neoprene casts. It is the intent of the present work to extend these findings to the functional characteristics of fluid dynamics of this area and then to determine their responsiveness to various pharmacological agents.

Methods: Albino rabbits are used. Red cells are obtained from the previously heparinized animals by cardiac puncture. The eyes are enucleated after sacrifice and kept in Krebs Ringer solution, containing 10% horse serum, and maintained at constant temperature. The eye is dissected, using a biomicroscope to isolate the iris, ciliary body and attached long posterior ciliary artery. The artery is then cannulated with a #30 hypodermic needle which is attached by means of polyethylene tubing to a bottle containing Krebs-Ringer solution, horse serum and red blood cells. The tissue specimen, in

a constant temperature bath, is then perfused and observations of the flow are made at x25 and x40 power.

The procedure is also amenable after removal of sclera and cornea to the observation of flow in the choroidal layer.

Movies and photographic plates are made for detailed study and documentation.

Major Findings: In albino rabbits, the ciliary processes extend upon the posterior surface of the iris and go beyond the area of the circulus arteriosus. Because of the lack of pigment, the blood vessels of the ciliary processes are visible, and because of their transparency, flow of red cells is easily observed and studied.

The blood supply of the processes is composed of a variable number of vessels, which usually have their origin from the arteries of the iris and ciliary body. These vessels enter the base of the processes at various points and branch either into a large number of small vessels or into a small number of larger vessels, either of which have diameters greater than the capillaries of the iris. On the dorsal portion of the process is one large vessel which begins at the tip to join the choroid.

At the beginning of arterial perfusion, one immediately sees red cells coming from the radial branches of the iris artery and into the anteriormost artery of the process, to then proceed into the large vessel at the dorsum and thence to the choroid. Immediately thereafter, in the majority of cases, flow is observed in the other arterioles and capillaries which goes to the dorsal vessel. The latter thus has the characteristic of a vein and may be called either a Marginal or Dorsal vein. Sometimes the anterior portion of the processes is shunted from the flow and in these cases the main circulation proceeds through an arterial vessel which goes to the middle or posterior portion of the processes and to an ancillary dilated capillary bed which communicates directly with the marginal vein.

The marginal vein, at the level of the orbiculus, divides into two branches, one superiorly and one inferiorly. The superior vessel divides into a capillary net which lies between the choroidal and iridiociliary vessels. The inferior vessel proceeds under the processes and joins the choroidal veins. The flow thus may be seen to proceed from the marginal vein to either or both of these vessels.

The anterior portion of the processes has a second mechanism of flow, which is not too often seen, made up of a large vessel which originates from the capillaries and proceeds under the process to the choroidal vessels.

Significance to Program of Institute: The knowledge of the blood circulation in the various portions of the anterior uvea might shed light on the interrelationship between the dynamics of blood flow and that aqueous humor formation.

Proposed Course of Project: Thus study will be continued to include the effects of various drugs such as arterenol, acetylcholine and acetazolamide on the vessels of the ciliary processes and iris and upon the dynamics of flow in this area.

Part B included Yes [ ] No [X]

PHS-NIH
Surgical Neurology Branch
Summary for Calendar Year 1960

During the past year, 234 patients have been studied in the ward environment, while 260 patients made 309 visits to the Outpatient Department. There were 73 consultations. In this group of patients, 118 came to operation, of which 41 were epileptics, 22 had intracerebral tumors without epilepsy, 20 suffered from involuntary movements for which stereotaxic procedures were performed, and 35 were operated on for a miscellany of lesions requiring surgical treatment. 23 reports were prepared for publication.

In the ward environment, the program in involuntary movements gained momentum, and the stereotaxic techniques were applied to a series of patients. Temporal lobe epileptics continued to constitute the majority of the ward population, which was completed by a small proportion of children with cerebral palsy and other developmental abnormalities. The various projects were represented during crowded outpatient days and all projects continued to receive increasing numbers of requests for admission.

The Branch was visited by Dr. Eben Alexander and Dr. Robert Johnson, as well as Mr. Murray Falconer and Dr. Wilder Penfield. Doctors Alexander and Johnson accepted and fulfilled appointments as Visiting Neurosurgeons. Each brought to the Branch samples of his professional experience which were very much appreciated. Dr. Alexander represents a Visiting Neurosurgeon from the academic lists, while Dr. Robert Johnson represents an alternate appointment drawn from the lists of private practice. Such alternation is characteristic of the Visiting Neurosurgeon Program. Dr. Wilder Penfield visited the Branch during his tenure as NIH Lecturer under the auspices of the Surgical Administrative Committee. He found time to visit all of the laboratories and for discussions on project material with various investigators. Mr. Falconer spent a month as a Special Consultant to the temporal lobe program. His clinical and clinical investigative experience which is so closely allied to our own was once more most profitable in its applications here. Dr. Alfred Luessenhop joined the Branch as Visiting Scientist, and then a Guest Worker. He is engaged in a study of cerebral vascular patterns which has clinical applications. The Branch also received brief and unscheduled visits from various neurologists and neurosurgeons who came from Warsaw, Chicago, Worcester, Vancouver, Paris, Mexico City, London, Vienna, Klagenfurt, Bern, Lausanne, Bogota, Nantes, Rehovoth, Basle, Berlin, and Colombo.

The Branch Chief wishes to express his gratitude to the Director, NINDB, and the Associate Director for their cooperation and enthusiastic support during the period covered by this report.

Dr. John Van Buren applied the stereotaxic apparatus which he has developed to patients suffering from lesions causing involuntary movements. As a result of such applications undertaken during the current year, he has

already reported the effects of deep stimulation in thalamus and parathalamic structures, and has completed treatment of some cases. While the effects of such stimulation in depth must await further knowledge for final interpretation, their observation constitutes a material contribution. The clinical effects of ablation (the end result of the emplacement, stimulation, and localization studies) have been gratifying in the small series of cases studied during the past year. In addition, Dr. Van Buren has observed the effects of depth stimulation in the temporal lobes, and particularly the effects of such stimulation in the mesial temporal structures in a group of patients suffering from temporal lobe epilepsy. These studies extend and confirm results obtained during the past year and have provided an additional diagnostic tool for the clinical analysis of the difficult bitemporal problem. In a series of 11 patients undergoing surgery for hypophysectomy, he studied the autonomic responses to electrical stimulation of the orbitotemporal cortex. Recordings of blood pressure, pulse, respiration, and esophageal and gastric motility were made. Electrical stimulation of the posterior orbital region and infundibulum yielded few, if any, responses, although mechanical stimulation of these areas seemed to yield profound change. He stimulated the junction of the anterior uncus and hippocampal gyrus and obtained well-defined responses. Blood pressure fell in most responses and a slowing of the pulse occurred. In some responses, however, there was an increase in blood pressure. Respiration was inhibited or arrested, while in 2 cases there was a definite increase in gastric motility. He has also completed an anatomical atlas which provides the fundamental background for the stereotaxic emplacement. In addition, his current anatomical studies include reconstruction of 2 normal retinae, 2 retinae derived from a case in which the chiasm has been interrupted by craniopharyngioma, and 2 retinae from a case of occipital lobe lesion. These studies have been fruitful in furthering the understanding of the ganglion cell layer of the retina.

Dr. Choh-luh Li reports on cortical intracellular potentials in response to stimulation of the geniculate body, basic mechanism of single cell discharges in the cerebral cortex, and basic mechanism of electrical activity recorded from the surface of the brain, as well as inhibitory interneurons in the cerebral cortex, and physiological studies in tissue culture material. He relates most of the electrical activity recorded from the brain surface to the cortical neuron, while he notes that the basic mechanism of single cell discharge in the cerebral cortex seemed quite similar to that observed in single muscle fibers. Briefly, he records evidence of an intrinsic, as well as extrinsic mechanism. The former is represented by rhythmic oscillation of the cell membrane, and the latter by small random potentials. He has begun the technical development necessary for the application of his microelectrode techniques in tissue culture media. This development must overcome serious difficultues, an example of which is the clogging of micropipettes by "imperfections" in the culture media. When these difficulties are overcome, the technique should provide interesting observations on in vitro cellular metabolism.

Dr. Igor Klatzo reports on pinocytotic activity in astrocytes, oligodendrocytes, microglia, and Schwann cells. In these experiments,

in vitro and in vivo preparations were studied. In vitro preparations were studied by cinemicrographic time-lapse records and by observations with fluorescence microscope on the characteristic pinocytotic uptake of fluorescein-labeled proteins. The glial cells have a characteristic inclusion in the area of edema in the white matter, as do the macrophages in the subarachnoidal space. Cats were injected with FLP before production of a localized edema in the white matter. These observations emphasize the importance of the glia as a metabolic intermediary. They also provide information concerning transmission of serum proteins in brain substance. He also reports on alterations in vascular permeability in experimental allergic encephalitis and in the brain of rats exposed to alpha particle radiation. In addition, he has reported on the pathology of Kuru Disease, the localization of myosin in human striated muscle by fluorescent antibody techniques, as well as a new method for quantitative study of precipitin reaction. He and his associates are participating in the projects on hypothermia and in the radio-frequency energy projects. He has studied the morphological changes which follow exposure of the brain to radio-frequency energy in the macaque. Also, he has studied the blood-brain-barrier in such animals using sodium fluorescein. He finds changes in the barrier in pons and medulla, with some extension laterally in the cerebellar hemispheres in acute experiments. In chronic experiments, only a few animals showed changes in blood-brain-barrier, and these were localized in the dorsal portions of the upper cervical cord. Other morphological changes in both acute and chronic preparations have been studied. The acute preparations show a striking tigrolysis in the large tegmental neurons of the pons, medulla, and the dentate nucleus of the cerebellum. Chronic preparations show changes in the dorsal region of the upper cervical cord. These are principally proliferative changes around small cystic cavities.

Dr. Anatole Dekaban presents a detailed report of 40 patients suffering the effects of lesions which would come under the general label of cerebral palsy. From this patient material, he observes that epilepsy of the idiopathic type can occur in infants under the age of one year. He concludes that in such a case, frequent daily attacks of petit mal and its variants may of themselves cause significant and irreversible mental retardation. He reports on maternal condition during pregnancy and subsequent course of birth which might relate to neurological abnormalities. He has reviewed 4,156 pregnancies. From this study, he postulates that various environmental factors acting during prenatal, intranatal and early postnatal life may be responsible for brain damage and the corresponding clinical sequelae in infants. He reviewed 15 brains of infants who died at the age of less than 3 days following cerebral birth injury and found 6 more types of hemorrhagic lesions. Similarly, he reviewed the outcome of 234 pregnancies in 48 diabetic women. He concludes that the fetal wastage in diabetic women is significantly greater than in the normal controls. He has also engaged in an anthropometic study in which he measures the external and internal orbital distance in males and females from birth to adulthood. He has made a total of 600 such measurements. Finally, he is producing congenital malformations in the nervous system in mice by ionizing radiation. In the course of this study, he has produced an atlas of the normal mouse

brain. The study presently includes data on what he calls "eleven age horizons of young mice". He has obtained 98 litters from irradiated mothers and 10% of these had major abnormalities, while 25% showed minor abnormalities. He is beginning to study chromosome patterns in patients with congenital abnormalities and mongoloidism. There are no definitive findings in this project at present, since it was begun recently.

Dr. Herbert Lansdell reports on psychological evaluation of temporal lobe disease. He notes that the effects of temporal lobe disease are not discernible by traditional intelligence tests. However, these parts of the brain seem to be involved in maintaining some balance in subtler functions tapped by verbal and perceptual tests where something is required that might be called the "judgment of the well adjusted person". Specifically, his tests note a slight decline in patients' abilities following unilateral lobectomy. These tests include the Gorham Proverbs, Pettigrew's Estimation Questionnaire, the MMPI, word association tests (which he invented), a test of visual perception (which he also invented), and Cattell's "16 Personality Factors" questionnaire.

Dr. Galindo has successfully applied a technique of deep hypothermia to more than 100 dogs. Working in conjunction with the Branch Chief, he has shown that esophageal temperatures in the range of 4 to 7° C. can be obtained and maintained with or without cessation of extracorporeal circulation. This technique has been applied in higher primates and it is now clear that it will provide a relatively safe background for intracranial and intracerebral procedures in the chimpanzee. A frontal lobectomy was carried out at 4° C. in a chimpanzee whose circulation had been arrested for 45 minutes. The esophageal temperature was 7° at the time of excision and the circulatory arrest was begun and continued in the temperature range 4 to 7° C. The animal recovered. This project may provide increased technical opportunity in the human operating room, as well as greater patient safety in after-care of devastating cerebral edema. The finding that a sympathetic block reduces or entirely prevents the chance of cardiac fibrillation is an important corollary, as is the finding that the heart may be selectively cooled so as to decrease the time required for cardiac arrest and thus further reduce the chance of fibrillation. Furthermore, the study seems to have demonstrated a temperature threshold for the primate hypothalamus or thermo-regulatory centers, below which there is no longer any irregularity in brain temperature and the electrical recordings from brain surface "stabilize".

In conjunction with the Branch Chief and Miss Lewis, Dr. A. S. Wiener typed, cross-matched, and otherwise analyzed blood characteristics of the chimpanzees in our colony. Results of this study showed that the majority of animals are in group A. All fall within the capabilities of the Lansteiner classification system and their corollary factors can be estimated by human techniques. The M-N factors have been derived from saliva experiments. The hematology of these animals is to be further investigated in conjunction with Dr. Undritz, hematologist at Sandoz Laboratories. Their unusual clotting capacity remains as a challenging target for hematological analysis.

Dr. Adamkiewicz, Dr. Bach, Mr. Pearlman, Miss Lewis and the Branch Chief have continued the studies on the effects of radio frequency. The apparatus has been adapted so as to study effects on the chimpanzee, as well as the monkey, and a new system of equipment has been devised so that the effects of radio frequency on serum protein targets can be observed. As a result of this year's work, it seems clear that there is a relationship between frequency and clinical or chemical effect. The chimpanzee responds to a frequency of 240, the monkey in the frequency range of 388. Electrophoretic changes have been produced in serum albumin in the range of 30 mc. and harmonics of this frequency. The dissociation constant of the solvent medium plays an important role in the protein changes during radiation. Protein in distilled water is not affected, while protein in buffered saline is most affected. The lethal effects of radiation on the whole animal were inadvertently demonstrated with the first application of the new transmitter to the chimpanzee. The animal died in less than a second. The changes in surviving animals have been carefully studied. Blood-brain-barrier changes continue to occur around aqueduct and upper fourth ventricle, while the morphology is characterized by tigrolysis localized in pons and medulla. Interestingly enough, aspiration or drainage of the spinal fluid protects the animal from the more severe clinical effects. The reason for this is not known.

Dr. Adamkiewicz and the Branch Chief are studying the effects of traditional electrical stimulation on the chimpanzee cortex. During this year, postcentral motor responses have been confirmed, and in addition the walls of the third ventricle and its floor have also been stimulated. This stimulation did not yield any responses observable by the usual polygraphic recording techniques. 532 responses of the human temporal cortex to electrical stimulation have been reviewed in a total of 883 evoked over all areas. These responses have been correlated and prepared for publication at this time.

Dr. Otenasek has studied the influence of cervical vagal stimulation on amygdaloid activity in the macaque. In 5 preparations, he has not detected any response in the amygdala.

Miss Lewis and the Branch Chief continued the investigation of the effects of hallucinogenic drugs on temporal lobectomized chimpanzees. Four derivatives of lysergic acid were tested, including psilocin. The temporal lobectomized animals do not respond to these drugs, whereas the normal animals do. In addition, these investigators continue to study the effects of cortical ablation, including the ablation of vocalization representations and temporal and parietal cortices. Bilateral parietal excision does not produce evidence of loss of right-left orientation, nor of unilateral ignorance.

Doctors Farrier, Laskowski, Maccubbin and Baldwin investigated the effects of low-sodium regime on patients with temporal lobe and other types of epilepsy. In the patient series studied during this year, there has been a significant reduction in seizure frequency during the low-sodium

regime. In the laboratory correlate of this project, discrete penicillin lesions whose activity was sustained at least 2 hours as recorded by localized spikes were exaggerated or perpetuated by intravenous injection of 10% sodium chloride. Moreover, all such lesions when allowed to subside and remain quiescent for a period in excess of 2 hours were rapidly reactivated and exaggerated so as to become diffuse and emerge rapidly into status epilepticus by injection of 10% sodium chloride.

Dr. Ferris and the Branch Chief have studied psychoses in temporal lobe epilepsy in 25 patients and noted a specific relationship in the onset of the psychosis, its severity, and the time and abruptness of withdrawal of anticonvulsant medication. The type of the psychosis was previously described and was characteristic. The Branch Chief continues the study of language in patients with temporal lobe disease and notes the relationship between this complicated abnormality and what Lashley called an abnormality in serial behavior. Dr. Maccubbin has reviewed the effects of temporal lobectomy in 88 patients whose operations were completed at least a year ago, and in some cases as long ago as 8 years. His analysis demonstrates a 38% cure rate, and a 75% improvement. He has also studied the relevant surgical anatomy, but it remains difficult to make correlations in functional anatomy. However, the pes hippocampus continues to show some relationship to recent memory functions.

Mrs. Thompson has reviewed 6,000 photographs of spontaneous temporal lobe seizures, and has prepared an exhibit of outstanding clinical characteristics. Mrs. MacDonald relieved Miss Lewis as Supervisor of the Neurological Operating Room in which the work on various technical devices has continued. With the assistance of Dr. Laskowski, draping, posturing, ventilation, lighting, and other instrumental techniques are under study. Miss Lewis has finally completed the Manual of Neurosurgical Techniques and Aseptic Techniques devised by her for the neurosurgical unit and which has, in large measure, been adopted by the Surgical Administrative Committee.

Serial No. NINDB-54(c)

1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: J. M. Van Buren, M. D., C. Ajmone Marsan, M. D., I. Klatzo, M. D., E. J. Laskowski, M. D., D. Maccubbin, M. D., P. Ferris, M. D., J. Gills, M.D., R. Farrier, M. D.

Man Years (calendar year 1960):
Total: 5.25
Professional: .25
Other: 5.0

Project Description:

Objectives:

a. To study causal mechanisms of epileptic seizures in man and other primates.

b. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.

c. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

d. To study brain function as it is exposed in the extravagant experiments devised by these lesions.

Methods Employed:

a. Clinical neurological examination
b. Special radiographic examination
c. Electrographic examination
d. Electrocortigraphic examination
e. Electrical stimulation of the lesion exposed at operation
f. Selective isolation of the lesion at operation
g. Photographic and sound recording
h. Histological and chemical examination

Major Findings: During the last year, 130 patients with cerebral seizures were studied in the operating room and on the ward. Of these, 70 owed their seizures to origins in one or both temporal lobes. On the ward, behavior, language, response to low and/or high sodium diets, and response to various anticonvulsant regimes were studied. In addition, 11 such

patients were studied by Dr. Van Buren with depth electrode techniques.

This is a time for summing up. The total number of major operations for focal epilepsy now stands at 300. There are 172 temporal lobectomies in this group. The results of operative treatment in 88 cases have been reviewed by Dr. Maccubbin and the Branch Chief. These cases have been followed for a period of 1 to 8 years after operation. Coincidentally, Mr. Savard has completed a social study of postoperative effects in 33 cases studied 5 years after operation. 38% of the larger group are seizure free, while 75% are considerably improved. The social study clearly shows improvement in family and community relationships, as well as economic usefulness. This is most gratifying, since all cases present serious behavioral problems before operation.

Unilateral intervention in the "bitemporal case" has proven rewarding to the patient and project. For example: A 20-year-old right-handed boy with temporal lobe seizures had "bitemporal" independent electrographic abnormality. There was some indication of predominance on the left. His seizure pattern was clinically related to the left temporal lobe, which was removed at operation. A glioma was found at removal, although contrast and other studies did not predict its presence. The boy has been free of seizures since and the right-sided abnormality is subsiding.

The surgical anatomy of temporal lobe epilepsy has been reviewed and summarized. It is clear that removal of the lateral cortex alone does not relieve the seizures. The removal (as previously predicted) must include mesial structures, but paradoxically can exclude pes. Removal of the pes hippocampus has been followed by serious disturbance in recognition and recollection. Excision of amygdalae does not produce discernible defect, but seems necessary for eradication of seizures in most cases.

The psychoses related to temporal lobe epilepsy have been studied. Dr. Ferris and the Branch Chief studied 25 cases characterized by overt psychotic episodes occurring during hospital study. These psychoses consist of dissociation and bizarre language, with hallucinations. One of these cases is exemplary. A 35-year-old mathematician with a doctorate in theoretical mathematics came to the ward environment because of seizures originating in the left temporal lobe. These were related to a left temporal glioma and a cicatrix subsequent to its excision in an operation performed by Harvey Cushing approximately 30 years ago. There were no signs of expansion or pressure. This man was mild mannered and moderately well spoken on admission, at which time he had not suffered any clinical seizures for several months. However, it was clear after careful interview examination that he had difficulties in language and was suffering from some thought disorder. He was interested in comparing religion to mathematics, to his illness, and perseverated on the theme that his illness no longer mattered because the anxiety attendant on it had been relieved by God in a recent mathematical revelation. Following withdrawal of anticonvulsant medication, the patient did not at first suffer any clinical seizures, but gradually became psychotic and could not establish contact with his environment. He then had one or two clinically discernible temporal lobe seizures. His

associative speech, evident thought disorder, depersonalization, and apparent hallucination, led the psychiatrists to characterize him as a schizophrenic. Following restitution of anticonvulsant medication, he did not have any further clinical seizures. Moreover, his language improved and he established contact with persons and places around him. There was considerably less evidence of thought disorder, and he discussed his illness in a rational and lucid fashion. It seems clear that persons with epileptic disease of the temporal lobe are prone to a schizophrenic-like psychosis. This psychosis is an exaggeration of the interictal behavior observed in all cases. It can be precipitated and may be dangerously exaggerated by withdrawal of anticonvulsant medication. Such psychoses are not observed in patients with epilepsy originating in other parts of the brain. There is a relationship between the development of the psychosis and the withdrawal of medication. If the affected lobe can be removed, the patient is no longer prone to such psychotic episodes.

Six thousand spontaneous ictal episodes have been photographed in the ward environment. The characteristics of hand, arm, foot, and leg movement in the seizure pattern have been studied. The facies of automatism have been catalogued, as have the more evident and more frequently reported movements of mouth and jaws. This work, carried on by Mrs. Thompson and staff, continues to constitute a valuable diagnostic and scientific element in the project.

Doctors Farrier, Laskowski, Maccubbin, and the Branch Chief have studied some of the effects of high and low sodium diet on seizure frequency. There seems to be some relationship between the frequency of attacks and the salt intake. Twenty patients have been studied in a protocol which compares the seizure frequency "on" anticonvulsant medication, or "off" anticonvulsant medication, to high and low sodium intake, with and without diuretic assistance. From this study, now only a year old, it seems that electrolyte regulation, insofar as this can be done by diet and by extradietary medication, plays an important therapeutic role.

Dr. Gills and the Branch Chief have studied the results of the Funkenstein test in 28 cases of epilepsy. Twenty-five of these were cases of temporal lobe epilepsy. The test results are as yet inconclusive.

In the laboratory, the study of penicillin-induced lesions continues. It is now possible to induce an epileptogenic lesion in the parietal cortex of the chimpanzee at operation (designed to expose the cortex of both hemispheres) and maintain the activity of this lesion in an area approximately 1 cm. square. Such lesions have been monitored locally and elsewhere across the cortex as described in a previous report. When such a chimpanzee is given 10% sodium chloride by vein while the discretely localized lesion is firing, the record of the firing becomes continuous instead of intermittent. Thereafter the firing spreads and the disturbance becomes diffuse. If such a lesion is allowed to become inactive so that discrete focal spikes are no longer recorded 2 hours after the creation and original recording of the lesion, it seems to be reactivated within 30 minutes after the intravenous

injection of 10% sodium chloride. Conversely, if the lesion is actively firing and has been actively firing for an hour, the firing seems to disappear and the record returns to normal if the animal is bled so as to lose 100 cc. of whole blood. The hypotensive effects of such a loss are buffered with Levophed which does not ordinarily affect the electrographic tracing, while tracings of the cortex during hypotensive states induced by other drugs do not show any change in the firing of the lesion.

A relationship between the penicillin lesion and local electrolyte changes is being sought through micro-incineration assay. Such a lesion, once created and delineated, can be excised and the resulting specimen subjected to micro-incineration techniques. As a further corollary to these studies of seizure mechanisms, Dr. Klatzo, Dr. Otenasek, and the Branch Chief are studying permeability of the blood-brain-barrier around the penicillin lesion by means of tagged albumin and ordinary fluorescent techniques. These fluorescent studies are correlated by similar ones now being undertaken in the human operating room.

Significance to Neurological Research: These observations may contribute towards further understanding of epileptic mechanisms as they occur in temporal lobes of higher primates.

Proposed Course of the Project: The various clinical and experimental studies will be continued, with the expansion of the electrolyte project.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Baldwin, M.: The problem of temporal lobe epilepsy in occupational medicine. AMA Arch. Indus. Med. (in press)

Crisp, L. R., Lewis, S. A., & Baldwin, M.: Universal stand with drape rack and instrument trays for neurological surgery. Am. J. Nursing (in press)

Serial No. NINDB-55(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Functional Representation in the Temporal Lobe of Man and Higher Primates.

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: John Van Buren, M. D., Shirley Lewis, R. N., Richard Otenasek, Jr., M. D., Joseph Adamkiewicz, M. D.

Cooperating Units: Neurology Social Service

Man Years (calendar year 1960):
Total: 10.25
Professional: 4.25
Other: 6.00

Project Description:

Objectives: To further understanding of functional representations within the temporal lobe of man and higher primates.

Methods Employed:
1. Electrical stimulation and recording of the human chimpanzee and monkey temporal lobes: (a) directly, after operative exposure; (b) indirectly by depth electrodes and scalp recordings.
2. Ablation of all or parts of the temporal lobes.
3. Anatomical studies of whole brain after temporal lobe excisions.

Major Findings: The studies on unilateral and bilateral ablation continue. During the past year, studies on the parietal cortex have been added. In one preparation (a chimpanzee) the parietal cortex has been ablated on both sides. Paradoxically, these ablations were not followed by the predicted "parietal lobe syndrome". This preparation will be developed by further ablation of the adjacent temporal cortices and the subsequent behavior compared to peers whose temporal cortices have been ablated on one or both sides without intervention on the parietal lobe. There is some evidence and a large amount of conjecture that the temporal and adjacent portions of the parietal cortex act in concert, and that this large associative area is, in fact, a complete functional unit. It is interesting that in one side of such a unit, speech is represented, where it is seldom

represented even in part in the opposite homologue. In some chimpanzees, the areas which ordinarily control speech in the human have been ablated. As expected, such ablation in the chimpanzee has not materially altered communication capabilities. However, this project is, as yet, incomplete and may yield further data if pursued.

The early results of summation of the 172 temporal lobe excisions carried out on humans indicate a change in affect, a change in language, and in some cases a change in sexual function. The moot question as yet quite unanswered is "do these effects relate to the area of removal and/or the lesion in that area for which the excision was carried out?" However, the social summary of Savard and the clinical summation of Maccubbin both indicate that there is a mildness and an amelioration in behavior in both the right and left-sided cases, and that the right-sided cases show improvement in language.

At this time, we have records of 532 responses to electrical stimulation from the temporal lobe. The number of secondary sensory responses continues to increase, whereas the number of so-called psychical responses from surface stimulation has declined almost to zero. None the less, there are 60 such responses in the series at this time. These responses will be correlated with those derived from the adjacent central and parasylvian regions, and in turn these data will be correlated with approximately 300 responses derived from stimulation of chimpanzee cortex and deep structures. The latter responses now clearly indicate that Sherrington's suspicion of the validity of postcentral motor response has been confirmed. The orbital and mesial frontal surfaces, as well as the brain stem structures, must be probed during the coming year. During the present year, Dr. Otenasek has investigated the suspected relationship between amygdaloid response and afferent vagal stimulation. Up to this time, he has not found evidence of a clear relationship.

During the latter part of this year, the effect of an electromagnetic field on cortical response to stimulation and cortical recording characteristics has been tested. A simple magnet designed for foreign body search in clinical surgery was used. This instrument has a "power range" of approximately 6 cm. Thirty seconds' "application" to a cortical area in the parietal lobe was followed by an increase in amplitude and an apparent increase in frequency in all relevant leads. These changes appeared approximately 50 seconds after the application. This interesting, but as yet completely unclarified, observational series will be pursued in the coming year in connection with the radio-frequency work.

The most interesting assumption or preliminary conclusion on temporal lobe function is based on the patients' observation that meanings are clearer after lobectomy. As noted in the clinical and social reports, the majority of patients describe with varying degrees of clarity a sense of increased interpretive acuity and greater spontaneity in appreciation of their surroundings. These suggestive observations are to some extent born out by observations of their peers and by professional observers. However, they

have not found any confirmatory assessment in the formal psychological testing.

Since there are now 172 cases of right or left temporal excision and over 532 responses to stimulation of the temporal region, as well as a thorough social study of 33 patients made at least 5 years after operation, and beginnings of a relevant and equally thorough clinical study, it seems advisable to contemplate the construction of a monograph as a means of compiling these data. Dr. Marsan has a very considerable number of tracings and there are some additional tracings from the laboratory and the chimpanzee work. Dr. Van Buren's data on depth stimulation and on the anatomical connections, and Dr. Dekaban's data on the embryological development would all seem to provide an adequate basis for such a monograph, which must include the psychological and psychiatric evaluations, as well as clinical studies of the seizure patterns resulting from the various lesions in the temporal lobe.

As a continuation of the study of effects of hallucinogens, certain chemical relatives of lysergic acid have been tested. The following 5 preparations, made available to us by the Sandoz Laboratories, have been given to the chimpanzees in the manner described in the report on the effects of lysergic acid: (1) 4-hydroxy-w-N, N-diothyl-tryptamin, (2) 6-hydroxy-w-N, N-dimethyl tryptamin, (3) rac. a-methyl-tryptamin-methansulfonat, (4) l-methyl-psilocin, (5) psilocin. Those animals whose temporal lobes had been removed did not react to these drugs. The intact animals did react. In general, such reactions may be compared to those obtained with lysergic acid as somewhat milder, but of the same quality. Psilocin is of particular interest because it produces hallucinations in humans without the concomitant panic and intense perceptual disorganization which characterize the effects of lysergic acid. Psilocin affects the chimpanzee so as to produce euphoria, mild disorientation, and confusion. It does not evoke the panic and severe perceptual aberration characteristic of the lysergic acid effects.

Dr. Laskowski and Dr. Lansdell have studied the lateralization of speech representation in the temporal lobe by means of the Wada test. As of the present, their results indicate that the majority of cases are lateralized to the left brain, irregardless of the so-called handedness. The test has also been applied in the EEG Laboratory as an adjunct to extant lateralization techniques.

Significance to Neurological Research: This project provides opportunity for study of the functional representations of the temporal lobe, as well as its structural relationship.

Proposed Course of the Project: This project is developing because of information derived from electrical, surgical, anatomical, and biochemical studies. It will continue development from this wide base.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Baldwin, M.: Pathophysiology of cerebral concussion. N. Y. State J. of Med. (in press)

Serial No. NINDB-56(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Involuntary Movements

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: C. Ajmone Marson, R. Hertz, H. Lansdell and T. Cornman

Cooperating Units: Branch of EEG; Branch of Endocrinology, NCI; Clinical Psychology and Physical Therapy, CC.

Man Years (Calendar Year 1960):

Total: 1.2
Professional: .4
Other: .8

Project Description:

Objective: Although investigation of the basic cause of abnormal movement syndromes in man is most desirable, the means of carrying out such an investigation, apart from recording the therapeutic effects of lesions within the basal ganglia in patients suffering from abnormal movement problems are rather few. Thus, interest at the present time is centered largely in observations which can be made with electrodes implanted at the sites planned for later ablation. Studies undertaken with these patients at the present time are as follows:

(1) Recording of reported sensory results of stimulation.

(2) Oscillographic recording of evoked activity at the cortical surfaces from stimulation within the lenticular nucleus and thalamus (with Dr. C. Ajmone Marsan).

(3) Oscillographic recording of areas of spread of electrical impulses from lenticular nucleus to thalamus and vice versa (with Dr. C. Ajmone Marsan).

(4) Recording of the autonomic effects of depth stimulation.

(5) Photographic recording of the motor effects of depth stimulation.

(6) EEG recording of activity in the depth under waking, natural sleep and drug induced sleep (with Dr. C. Ajmone Marsan).

(7) EEG recording of the effects of lesions in the depth upon the electrical activity recorded from the scalp (with Dr. C. Ajmone Marsan).

(8) Evaluation of the endocrinological effects of lesions within the basal ganglia (with Dr. Roy Hertz).

(9) Analysis of the psychological effects of lesions within the basal ganglia (with Dr. H. Lansdell).

(10) Practical physiological effects of the therapeutic ablations as judged by standardized motor testing procedures (with Dr. Tillye Cornman).

Method Employed:

Since all the physiological data must be related to an anatomical substratum considerable time has been spent in the preparation of a variation atlas of the human brain by means of gelatin imbedded hemispheres sectioned in the sagittal plane at 1-2 mm. thickness. After each section has been cut the remaining block was photographed under a grid aligned according to the lateral projections of the anterior and posterior commissures. In this way, the atlas in the sagittal plane could be made up for each of the 25 hemispheres. Based on this method a coordinate system for recording all points of stimulation and ablation has been devised and all physiological work can be related to this anatomical system.

Major Findings:

To date, 8 patients have been subject to depth electrode placements and ablation in the basal ganglia. Patient material has been 2 choreoathetotics, 3 dystonics and 3 patients with Parkinson's syndrome. Multiple port depth electrodes have been placed in the thalamus and/or pallidum in these patients as well as an extradural electrode over the frontal cortex.

The clinical results have been encouraging and have demonstrated that the method can be carried out without significant morbidity. By the use of multiple port depth electrodes, it has been possible to study 98 points in the anterior inferior thalamus, genu of the internal capsule, both segments of pallidum, putamen and caudate nucleus in these 7 patients.

Preliminary analysis has only been carried out with the sensory responses, although, to our surprise, sensory responses have been obtained in nearly all subjects. Practically all of these were

referred contralaterally to the side stimulated. If the placement is near the optic tract, (as with the placement in the medial pallidum) visual sensations of stars may be seen contralaterally or straight ahead of the patient. More rarely, (2 patients) formed objects have been seen (clouds and spirals). Somatic sensation has consisted in feelings of tingling, tickling or warmth referred to various portions of the contralateral body. Epigastric sensations of nausea or hunger have been elicited from electrodes passing into the mesial temporal region and from electrodes lying in the pallidum.

No systematic analysis of other data obtained to date has been made. However, preliminary observations indicate that brief shocks within the medial segment of the pallidum have a rather wide distribution in the thalamus (ventralis anterior and ventralis lateralis) while conduction of shocks originating in the inferior portion of the ventralis lateralis go only to the mesial segment of the globus pallidus.

Significance to Neurological Research:

The use of depth electrodes in regions of the basal ganglia proposed as sites for later therapeutic ablation offer the opportunity for variety of neurophysiological studies of the depth areas of the human brain. The use of detailed human brain atlas permits some degree, at least, of anatomical-physiological correlation. Through these studies it is hoped to learn more about the physiological alterations underlying syndromes of involuntary movement and in addition, data will become available regarding other aspects of the physiology of the human basal ganglia.

Proposed Course of Project:

Data will be collected on each patient and each of the foregoing categories mentioned under Project Description.

Part B included Yes

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Van Buren, J. M.: Sensory, motor and autonomic effects of mesial temporal stimulation in man. J. Neurosurg. (in press)

Van Buren, J. M., Bucknam, C. A. and Pritchard, W. L.: Autonomic representation in the human orbito-temporal cortex. Neurology (in press)

Van Buren, J. M. and Ajmone Marsan, C.: A correlation of vegetative and EEG components in temporal lobe epilepsy. J. Neurosurg. (in press)

Serial No. NINDB-57(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of Lesions upon the Function and Structure of the Human Central Nervous System

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: None

Cooperating Units:

Man Years (Calendar Year 1960):

Total: .6
Professional: .2
Other: .4

Project Description:

Objective: This study has a dual aim: (1) to carry out physiological-anatomical correlations in man, (2) to evaluate the effects of new methods of treatment of tumors of the central nervous system. In the present program attempt will be made to utilize the intrusion of disease upon the central nervous system of man as an "experimental" lesion.

Methods Employed:

Specifically, the material is used in three major ways: (1) physiological observations can be made during or after surgery, (2) the effects of surgery itself can be evaluated, (3) post mortem material may, in certain cases, prove valuable for anatomical studies.

Major Findings:

(1) Responses from stimulation of the mesial temporal region in man.

In a series of 11 patients subject to epilepsy of the temporal lobe, depth electrodes were implanted in the mesial temporal regions. In each instance, the relationship of the electrode to the tip of the temporal horn was visualized pneumographically so that anatomical plots of the electrode position could be made. The electrodes were left in place 1-2 weeks and the effects of depth stimulation were observed clinically, photographically and with special apparatus recording autonomic function.

The latter apparatus recorded blood pressure, skin temperature, EKG, skin resistance, plethysmogram, esophageal and gastric motility and respiration.

The areas in which stimulation resulted in a clinical automatism were clustered close to or within the amygdaloid nucleus, and the pes hippocampus. Autonomic effects with the automatisms were invariable and included hypertension, tachycardia (rare bradycardia), fall of skin resistance, reduction in the amplitude of plethysmographic oscillations, swallowing with repeated esophageal peristaltic waves (rarely inhibition of gastric motility), and inhibition of respiration or expiratory apnea. In several cases, patients responded appropriately during and after a stimulation leading to an automatism yet later became unresponsive. Emotional responses were few. Fear was noted in 2 apprehensive subjects but not in those who were previously at ease. In 1 patient laughter appeared with stimulation and was reproducible as a part of an automatism.

Autonomic changes, apart from those seen with stimulations inducing an automatism were relatively few (fall of skin resistance and respiratory inhibition were most frequent) and were usually accompanied by a subjective sensation of visceral or poorly localized somatic nature. Epigastric sensations were most frequent, sensory responses, appeared from electrode placements well outside the amygdaloid complex and were also seen from placements in the region of the substantia innominata and the pallidum. The proximity of these regions to the inferior thalamic peduncle and other systems running medially from the anterior temporal region may be noted.

The responses proved variable and often were not reproducible despite close monitoring of the stimulation current. In one case progressive fatigue of the response of the region from which automatism could be elicited was observed during two separate periods of stimulation.

(2) Functional correlations between the clinical, autonomic and electrographic features of temporal lobe epilepsy (with Dr. Ajmone-Marsan).

A series of 21 ictal episodes associated with metrazol injection were observed in 20 patients presenting seizures characterized by visceral phenomena, automatisms and predominantly temporal electroencephalographic abnormalities. Records of blood pressure, skin temperature, EKG, skin resistance, esophageal and gastric motility, respiration and the notation of the level of responsiveness were made simultaneously with a 16 channel EEG recording.

Autonomic recording gave evidence of a remarkably stereotyped response consisting predominantly of hypertension, tachycardia, fall of skin resistance, esophageal peristalsis, inhibition of gastric motility and inhibition of respiration. Variations in this pattern lay predominantly in the failure of a given change to appear or in the sequence of the changes. In nearly all instances, however, failure of the patient to

respond was preceded by some alteration in autonomic function.

Most of the aurae were reported when no significant electrographic activation had occurred and many autonomic changes appeared without an electroencephalographic correlate. No autonomic or clinical change was seen to accompany the onset of the focal ictal activation either on the surface or in the depth of the temporal lobe, although such changes might appear later as the activity increased in extent and amplitude. The bilaterally symmetrical roughly synchronous bursts of activity usually of greater amplitude in the frontal regions might or might not be accompanied by autonomic change. No change was seen in the level of responsiveness with these brief bursts. Sudden generalized loss of voltage of the record formed a constant warning of imminent clinical seizure activity in 6 cases either as an automatism or a generalized convulsion. Most of the changes in the level of response or of autonomic function appeared against a background of gradually increasing generalized epileptic activation with or without a preponderance in the temporal region. Under these circumstances, various phenomena including loss of responsiveness occurred without evident correlation in the electroencephalographic record.

(3) The orbito-temporal cortex of 11 patients undergoing pituitary surgery for the palliation of metastatic cancer was studied for autonomic response to electrical stimulation. General anesthesia was used and recordings made of blood pressure, pulse rate, respiration, esophageal and gastric motility and the pneumo-plethsymogram. Responses from the posterior orbital region and from the infundibulum of the hypothalamus were meager, although mechanical stimulation coincident with section of the hypophyseal stalk might yield profound changes.

An area on the anterior uncus at its junction with the hippocampal gyrus produced well defined constant responses. The blood pressure usually fell coincident with bradycardia but might rise. Respiration was inhibited or arrested. Arrest might occur at any stage in the respiratory cycle. Esophageal contractions appeared which might follow or be independent of the laryngeal component of deglutition. In two instances, a well defined increase in gastric contractions occurred. No changes were noted in the plethysmogram. The similarity of this complex of response to that elicited by afferent stimulation of the superior laryngeal nerves and vagi in lower animals was noted and discussed.

Proposed Course of Project:

As the anatomical material becomes available in patients subject to pituitary stalk section in the palliative treatment of metastatic breast cancer, anatomical-functional studies will be carried out on these patients in cooperation with the National Cancer Institute (Dr. Roy Hertz).

In a few future cases of hypophysectomy in ovarectomized patients, it is planned to study the mitotic activity of the normal human pituitary

by preoperative injection of Tritium labeled Thymidine. This will be of importance in estimating the mitotic activity of the normal pituitary gland, its distribution and cell types involved in such activity.

A study is planned for enumerating the normal population of the supraoptic and paraventricular nuclei in the human hypothalamus, then relating changes in this population following hypophyseal stalk section and hypophysectomy to the patient's subsequent water balance.

Part B included No

Serial No. NINDB-58(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of the Functional Anatomy and Pathology of the Human Visual System

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: None

Cooperating Units:

Man Years (Calendar Year 1960):

Total: 1.2
Professional: .4
Other: .8

Project Description:

Objective: This study is directed toward a better understanding of the correlation of form and function within the human visual system. It is intended to carry out these studies correlating clinical physiology with anatomy using the best quantitative methods available. At the present time, the study has been divided into three parts:

(a) Retina

(1) Study of the normal human and primate retina using the Golgi and chromatic methods.

(2) Study of the effects of the lesions of the optic pathways upon the retina.

(b) Study of the effects of lesions of the optic pathways upon the lateral geniculate body.

(c) Study of the visual field defects following temporal lobectomy.

Methods Employed:

The methods employed have been given in detail in report for Calendar Year 1957 and 1959.

The present material in serial section on hand is as follows: 11 human cases with normal visual fields and normal retina, 1 normal monkey and 1 normal chimpanzee; 8 human cases and 1 monkey with lesions of the optic radiation; 5 monkeys with lesions of the optic tract; 7 patients with lesions of the chiasma or pre-chiasmal visual system; 2 monkeys with unilateral section of the optic nerve, and 1 patient with amblyopia exanopsia.

Since the collected material at the present time seems adequate, reconstruction studies of the topographical anatomy of the retinal ganglion cell layer have been started. These studies will entail indicating on 15X photographs of each retinal section, the position of each ganglion cell over 15 micra in diameter and indicating on the photograph the margin of various thicknesses of ganglion cells from one to five cells thick. The original slides are then enlarged 40 diameters, the retina sketched and the landmarks indicated on this tracing. The distances from a central marking cut in the retina to all landmarks are then measured by a curvilinear map measuring device. Then the measurements are transferred to a 10X plot of the retinal ganglion cell layer and the outlines of this layer are gradually built up as the serial sections are individually spotted, enlarged and measured.

Major Findings:

To date, reconstruction has been carried out on both eyes of 1 individual with normal retina, 1 patient with virtual transection of the chiasma by craniopharyngioma and 1 patient with a metastatic choriocarcinoma to the occipital lobe producing an inferior homonymous quadrantanopia.

(1) It has been found that the topographical arrangement of ganglion cells in the normal retina is not circular around the macula but forms an elongated oval with its long axis horizontal. The thinner the ganglion cell layer, the greater the ratio of long axis to short axis of the oval. In the thickest portions of the ganglion cell layer the margins of a layer of given cell thickness tend to progressively approximate a circle. However, even with ganglion cell layers of 5 cells thick the oval has the ratio of about 13:10 with the long axis horizontal.

(2) Although the optic nerve head appears circular, the actual opening in the ganglion cell layer is an oval with its long axis vertical.

(3) Ganglion cells with a soma of over 15 micra tend to have the highest population at the immediate margin of the densely populated portions of the ganglion cell layer (1 cell thick and over).

(4) In the case of craniopharyngioma in 1 eye a maximum ganglion cell thickness in the parafoveal area of 4 cells permitted a visual

acuity of 20/20. In the other eye with a maximum parafoveal cell layer of 3 cells thick the visual acuity was only 20/100. Since the distance between the blind spot and the macula was known both in degrees of visual angle and in millimeters of retinal surface, the visual fields and the anatomical plots of the topographical ganglion cell anatomy can be superimposed on the same chart. It was noted that isopters of 5/2,000 white and larger could be noted by the patient outside the portion of the ganglion cell layer more than one cell thick. This patient had a striking bitemporal visual field defect and there was outstanding degeneration of the retina in both of the blind fields.

(5) In the patient with an occipital choriocarcinoma and a homonymous inferior quadrantanopia, there was demonstrable degeneration of the large ganglion cells in the blind quadrant and a depression of the margins of the ganglion cell plots in this region, i.e., the margins were closer to the fovea in the blind area than elsewhere.

(6) Initial survey of a monkey retina who had an occipital lobectomy 4 years before being killed shows that this transneuronal degeneration of the retina of retrograde character can be exceedingly distinct if enough time is allowed. In the case of the monkey, the parafoveal degeneration on the blind side was shown by the retention of a ganglion cell layer of only 2 cells thick as opposed to the normal 7 to 8 ganglion cells on the intact side. As far as the investigator is aware, this transneuronal degeneration in the visual system has not previously been reported.

Significance to Neurological Research:

The topographical anatomy of the ganglion cell layer of the retina has never been worked out by means of reconstruction studies. The present efforts are directed toward investigation of the normal anatomy and the effects of lesions within the visual system.

Proposed Course of Project:

The topographical anatomy will be worked out primarily in man with supporting evidence on chimpanzee and monkey material. With this background the effects of destructive lesions in the visual system will be evaluated. Time factors seem very important for this degeneration and particular attention will be paid to these. A later study will entail the reconstruction of retinae subject to papilledema in an attempt to determine better the chronology of retinal degeneration following this lesion. Since the visual fields of most cases had been examined by the investigator prior to death, correlation between the anatomical findings and the visual fields will be made in all cases where this is possible.

Part B included Yes

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Van Buren, J. M.: A projection apparatus for human neuroanatomical reconstruction. Anat. Rec., 136: 363-366, 1960

Serial No. NINDB-59(c)
1. Surgical Neurology Branch
2. Primate Neurology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of Radio-Frequency Energy on Primate Brain Mechanisms.

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Sven A. Bach, M. D., Shirley A. Lewis, R. N., Igor Klatzo, M. D., Joseph Adamkiewicz, M. D., William Pearlman

Cooperating Units: Instrument Engineering & Development Branch

Man Years (calendar year 1960):
Total: 5.25
Professional: .25
Other: 5.00

Project Description:

Objectives: To study the effects of radio-frequency energy as applied to the heads of living monkeys and chimpanzees.

Methods Employed:

1. Electronic

a. Development of transmitter series of physical capabilities for applying the energy to the target areas.

b. Further research and development of electronic devices so as to produce pulsing and modulating which will permit rapid application of peak power free of carrier wave dependence.

c. Development of panoramic analyzers sufficient for frequency analysis and record of other wave characteristics.

d. Development of signal generator and connections capable of introducing critical frequencies without minimal thermal energies into tissue culture medium.

e. Development of remote recording devices capable of telemetering electrographic outputs activated in an experimental animal by photic driving.

f. Orthodox electrographic recording of the effects of radio frequency.

2. Photographic recording of clinical effects.

3. Histological and chemical examination techniques.

Major Findings: During this year, a kilowatt transmitter has been obtained. We have acquired a capability for pulsation of transmissions and have devised resonant cavities of several sizes. In addition, the "chimpanzee chair" has been modified and adapted for use with the appropriate resonant cavity.

Once more, the monkey was subjected to the effects of radio-frequency energy, and in a series of 25 animals, the results obtained during the previous year were confirmed. Then a chimpanzee was seated and positioned with his head in the resonant cavity previously used for the monkey. He was radiated at the frequency of 390 mc., which was considered critical for the macaque. Despite relatively long periods of radiation, he did not show any response whatsoever, save a rise in temperature accompanied by profuse sweating. He remained alert, responded to vocal and other auditory stimuli, and his neurological examination was considered normal. He was radiated for a maximum period of 30 minutes without any particular changes. Then a resonant cavity was applied so that we might radiate the chimpanzee at a frequency of 240 mc. The animal was positioned in this area as before and transmission begun. Unfortunately, this animal died just as the last relay was "going in" on the transmitter. The time of radiation probably was in the order of one second. Another chimpanzee was introduced into the area and this time the power at the source was decreased from approximately one kilowatt to approximately 300 watts. This chimpanzee had dilatation of the pupils and a paradoxical pupillary response, vocalization, grimacing, altered awareness, reflex changes, and bilateral weakness similar in quality and extent to the signs observed in the monkeys during radiation. In further experiments performed with greater control and better preparation, it was determined that the chimpanzee does not respond within the frequency range 380-390 mc., but responds rapidly and sometimes critically in the frequency range 240-280 mc.

A monkey was radiated at 388 mc. in the appropriate apparatus and with the previous technique. He developed the panoramic responses described in the past report. Then spinal fluid was drained away by lumbar and cisternal puncture. He was replaced in the area and radiated as before. He did not show any evidence of reaction or response to the radiation after the cerebral spinal fluid had been evacuated. This situation was reproduced in 4 animals with similar results. Thereafter, it was assumed that the spinal fluid had some effect on the site of action and the extent of damage produced by the radiation. Consequently, a study of the radiation effect on solutions of electrolytes and proteins has begun.

It has been possible to alter the electrophoretic pattern of human serum albumin by radio-frequency energy in the range of 30 mc. More

recently, it has been demonstrated that this effect is not dependent on thermal change, but seems related to the dissociation constant of the solvent medium. When the protein is radiated in saline, in physiological saline, or in buffered saline the electrophoretic pattern is changed in various ways. However, after radiation in distilled water, the electrophoretic pattern of the protein is not changed as compared to control values.

The morphological changes following radiation of monkeys' brains in the range of 388-400 mc. have been studied in conjunction with Dr. Klatzo. He reports it in detail elsewhere. In addition, the changes in blood-brain-barrier following such radiation in the monkey have received further attention. These changes continue to occur along the fourth ventricle and lower third of the aqueduct as in previous experiments.

Research and development under the guidance of Mr. Pearlman, and with the assistance of Mr. Edwards, has yielded several efficient and essential arrangements of equipment. There is now the capability for measuring power in the field and at the source, and an excitor stage consisting essentially of a 100-watt transmitter serves to activate the kilowatt transmitter. The signal characteristics in the field and at the source can be monitored. The field characteristics have been extensively studied using phantoms. As a result of this study, the antenna has been modified in form and position, and 2 variable resistance rods have been added to it. This provides for greater flexibility in loading. As a result of this development, we can now approach the coming year from a sound basis in physical analysis of field strength and with a very considerable flexibility in available equipment.

Significance to Neurological Research: This project explores the effects of electromagnetic "ultra-high-frequency" fields on living primate nervous systems. In so doing, it provides an opportunity for use of relevant energy forms as stimulation media in neurological experiments, and it may provide more basic information concerning the relationship between electromagnetic fields and cerebral function. Ultra-high-frequency radiation of protein complexes has already yielded evidence that these can be changed following such radiation and that the changes are specific and athermal in origin. In addition, the project demonstrates that micro-organisms, such as escherichia coli, produce mutant forms when subjected to this type of radiation. It may be possible to influence body fluids and infecting agents which act on the nervous system as a result of these experiments.

Proposed Course of the Project: Further study of effects and biophysical mechanisms of microwave irradiation on higher primates. Extension of radio-frequency application to tissue culture and other proteins and molecules in order to evaluate possible immunologic significance.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Baldwin, M., Bach, S. A., Klatzo, I., & Lewis, S. A.: Further observations on the effects of radio-frequency energy on the primate brain. J. Neuropath. & Exp. Neurol. (in press)

Serial No. NINDB-60(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of Inhibitory Interneurons in the Cerebral Cortex.

Principal Investigator: Choh-luh Li

Other Investigator: Shelley N. Chou

Cooperating Unit: None

Man Years (calendar year 1960):
Total: .6
Professional: .2
Other: .4

Project Description:

Objectives: It has long been suggested that some of the nerve cells in the cerebral cortex are inhibitory in function. Recently inhibitory interneurons were demonstrated with intracellular microelectrodes in the spinal cord. The purpose of this study is to demonstrate the existence of these neurons in the cortex.

Methods employed: Micropipette recording electrodes were inserted into the interior of cortical neurons of various areas in the cat. The changes of potential of the cell were compared and analyzed.

Major Findings: It was often observed that hyperpolarization and depolarization of two neurons closely located under similar experimental conditions. For example: a cell became depolarized and excited following a transcollosal volley, whereas another just next to it would be hyperpolarized in response to the same stimulation. This phenomenon was observed in the motor area, the somatosensory area and the visual areas. It was concluded that this can serve as direct evidence for the existence of inhibitory interneurons in the cerebral cortex. From the result of these experiments it was further suggested that the inhibition of responses of certain cortical neurons to an electrical stimulus is due to either a depression of the facilitation interneurons or an activation of inhibitory interneurons, dependent upon the accessability to the stimulus. Likewise, the inhibition of responses of certain cortical neurons to a given chemical agent may be determined by the agent's capacity for either depressing the facilitatory interneurons or activating the inhibitory interneurons.

Significance of program to Institute: The implication of the results of this study is to clarify some of the hypothesis proposed by other laboratories that certain pharmaceutical agents are inhibitory and certain others excitatory in action on the cerebral cortex. The hypothesis quoted has been further used to support another hypothesis that the waves of potential recorded from the surface of the brain are generated from certain histological segments of the nerve cells in the cortex. The results of the present study point out the importance of some basic knowledge of the cells before a hypothesis of cell behavior in general is proposed.

Proposed Course of Project: To continue studying the basic mechanism of electrical activity recorded from surface of brain.

Part B included Yes ☐ No ☒

Serial No. NINDB-61(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cortical Intracellular Potentials in response to Stimulation of the Geniculate Body.

Principal Investigator: Choh-luh Li

Other Investigator: Shelley N. Chou

Cooperating Unit: None

Man Years (calendar year 1960):
Total: .6
Professional: .2
Other: .4

Project Description:

Objectives: The visual responses recorded from the cortex has been of great interest since 1933 and no agreement has been arrived at in regard to the components constituting the various components of the responses. In recent years unit activity in the cerebral cortex has been studied with extracellular microelectrodes with which an inhibitory mechanism in the visual system affecting the activity of the cortical neurons in the visual cortex was suggested. Since the response of a neuron can be indicated by a change in its resting membrane potential and the inhibitory mechanism can be closely related to an increase in the resting membrane of the cell, an attempt has been made to record these changes of potential inside from the neuron.

Method employed: Evoked responses from the striate cortex of cats were recorded following single electrical stimulus applied to the lateral geniculate body. The responses were simultaneously registered with surface recording electrodes and intracellular electrodes in the grey matter of the cortex.

Major findings: The outstanding feature of these experiments was hyperpolarization following initial depolarization and excitation of the cortical cell membrane following stimulation of the lateral geniculate body. On the basis of our previous observations it would suggest that the stimulation activated the excitatory fibers as well as the inhibitory fibers. It was further inferred that there are many fibers specifically inhibitory

in function, projecting from the lateral geniculate body to the visual cortex. The inhibitory mechanism in visual function may be more important than in somatosensory function since the observations obtained in the somatosensory cortex in similar experiments were usually characterized by depolarization instead of hyperpolarization. The results of these experiments in visual responses also indicate that cortical neurons could be coincident with the time when the small deflections in the surface-positive wave occurred. This information would seem to rule out the argument that the small deflections are due to the arrival of impulses along the geniculo-striate fibers. Finally the difficulty in obtaining intracellular recordings in the striate cortex suggests that the cells in this cortical area are much more fragile than the cells in other cortical areas.

Significance of program to Institute: The demonstration that there is a strong inhibitory mechanism in the geniculo-striate system may throw light on the understanding of visual function.

Proposed course of project: Further study in connection with thalamic stimulation.

Part B included Yes [x] No [ ]

Serial No. NINDB-61(c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Publication: Li, Choh-luh and Chou, Shelley N. Cortical Intracellular Potentials and Their Responses to Stimulation of Lateral Geniculate Body. J. Neurophysiol., 1960, 23: 000-000.

Serial No. NINDB-62(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Basic Mechanisms of Electrical Activity Recorded from Surface of Brain.

Principal Investigator: Choh-luh Li

Other Investigator: Joseph Miller

Cooperating Unit: None

Man Years (calendar year 1960)
Total: .6
Professional: .2
Other: .4

Project Description:

Objectives: The knowledge on this subject can be summarized by a paper by Li and Jasper: "After over twenty years of study, the neurophysiological basis of the rhythmic oscillations in electrical potentials which can be recorded from the cerebral cortex remains poorly understood." Recently it has been the general thought that this activity is generated from dendrites. The present project is to test this hypothesis and further explore the basic mechanisms of brain waves.

Method Employed: Glass micropipette electrodes were used to record changes of potentials from the inside of single cortical nerve cells. These cells were spontaneously discharging or activated by various inputs.

Major Findings: On the basis of studies of spinal cord nerve cells and our studies of the cerebral cortex, intracellular dendritic potentials were difficult to record. The observations on which this study was based appeared to be obtained from activity recorded from nerve cell bodies. Unless other inference is made, the observations of this study indicate that the brain waves are closely related to activity of nerve cell bodies. The properties of cortical neurone were found to be different from those of spinal cord neurons in that the time course of repolarization of the neurons was found much longer although their liminal depolarization levels were almost the same. The prolonged repolarization following a discharge of a surface-negative wave (or the superficial response of Adrian), which has so far been thought to be

due to activity of dendrites in the superficial layers of the cortex, was found to be always accompanied by depolarization and excitation of cortical neurons. The surface-positive wave (deep response of Adrian), which has so far been believed to be due to activity of cortical neurons and deep dendrites, was found to occur with absence of neuronal activity recorded with intracellular microelectrodes.

Significance of program to Institute: The results of this study is not consistent with the current concept that brain waves are largely attributed to dendritic potential. It is important to note that no successful experiment has ever been made in isolation of dendrites in the cortex. From the available information, the results of this study would indicate that most of the electrical activity recorded from the surface of the brain can be closely related to the activity recorded from cell bodies of cortical neurons.

Proposed course of project: To continue exploring the basic mechanism of brain waves.

Part B included Yes ☐ No ☒

Serial No. NINDB-63(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Basic Mechanisms of Single Cell Discharges in Cerebral Cortex.

Principal Investigator: Choh-luh Li

Other Investigators: Shelley Chou and Joseph Miller

Cooperating Unit: None

Man Years (calendar year 1960):
Total: .6
Professional: .2
Other: .4

Project Description:

Objectives: In order to understand the underlying mechanisms of waves of potentials recorded from the surface of the cortex it is essential to know how the nerve cells in the cortex work. There have been numerous hypothesis about the surface waves of potential but none has direct support by convincing evidence. The present study is a first step in exploring this problem.

Methods Employed: The change of potential inside a single cortical neuron was recorded with a glass micropipette electrode simultaneously with a surface recording electrode. The activity was observed with the neuron at rest or under the influence of afferent volleys from various sources.

Major Findings: The basic mechanisms of single cell discharges in the cerebral cortex were found to be very similar to those observed in single muscle fibers. In brief, there are the intrinsic mechanism and the extrinsic mechanism. The intrinsic mechanism is manifested by rhythmic oscillation of the cell membrane and the extrinsic mechanism is manifested by random small potentials. These two mechanisms can be disturbed by either a change in the outside environment or supposedly a change in the metabolism of the cell. For instance when a cortical neuron was made epileptic there was a decrease in the resting membrane potential and an increase in the rhythmic oscillating potential.

Significance of program to Institute: This study aims at the understanding of surface waves of potential and of epilepsy in man.

Proposed Course: Further study along this direction is to be made.

Part B included Yes [x] No [ ]

Serial No. NINDB-63(c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards and Publications

Honors: Invitations to deliver lectures extended by University of Chicago and Duke University in July 1960.

Publications: Li, Choh-luh. Basic Mechanism of Single Cell Discharges in the Cerebral Cortex. Epilepsia, 1960 (in press).

Serial No. NINDB-64(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland

PHS-NIH.
Individual Project Report
Calendar Year 1960

Part A

Project Title: Physiological Studies in Tissue Culture Material.

Principal Investigators: Choh-luh Li and Igor Klatzo

Other Investigators: Joseph H. Miller

Cooperating Units: None

Man Years (calendar year 1960):
Total: .6
Professional: .2
Other: .4

Project Description:

Objectives: To investigate the responses of nerve cells and glial elements explanted in tissue culture has the advantages of direct visualization and control experimental conditions. It has been known that certain pharmaceutical agents are capable of rendering certain nerve cells epileptic, and others suppress epileptic activity. How about the glial cells? It is the purpose of this study to examine under direct vision the activity of these cells.

Methods Employed: Tissue cultured materials were obtained according to the method of Klatzo. The electrical properties of the individual elements under the microscope were studied with intracellular micropipette electrodes. The electrode was also used for injection into the interior of the cell with various drugs and for electrical stimulation by passing current through it.

Major Findings: Tremendous difficulty has been encountered mainly due to the imperfection of the culture media which clog the tips of the micropipettes. The fragmentary observations so far obtained simply confirm the findings reported in the literature.

Significance of Program to Institute: This study will throw some light on the mechanisms and treatment of epilepsy.

Proposed Course: To continue study of tissue culture cells.

Part B included ... Yes ☐ No ☒

Serial No. NINDB-65(c)
1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effects of Deep Hypothermia in Higher Primates

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Anibal Galindo, M. D., Joseph Adamkiewicz, M. D.

Man Years (calendar year 1960):
Total: 6.25
Professional: .25
Other: 6.00

Project Description:

Objectives: To study the effects of temperatures in the range of 4 to 7° C. on living chimpanzees.

Methods Employed:

1. Femoral and cardiac catheterization.
2. Extracorporeal circulation and cooling.
3. Deep anesthesia.
4. Polygraphic recording of blood pressure, pulse, respiration; brain, esophageal, and rectal temperatures.
5. Electrocorticographic recording.
6. Photographic recording.
7. Histological and chemical examinations.

Major Findings: Three chimpanzees have been subjected to a composite technique designed to provide extracorporeal cooling and sustenance at temperature levels of 4 to 7° C. These animals received moderately deep halothane anesthesia and artificial respiration above 25° C. (as recorded from esophagus), with 95% oxygen, 5% carbon dioxide, and then pure oxygen. Each was given 10 mg/Kg. of Quinidine and a sympathetic block. Perfusion and cooling began with a partial arteriovenous shunt. After the lowest temperature was obtained, a partial veno-arterial shunt was used to maintain circulation. These shunts were made by combination of femoral, arterial, and venous catheters.

This technique has so far provided for rapid cooling and warming. The average cooling time has been 20 minutes; that of warming, 30 minutes. The average time at 4 to 7° C. has been 2 hours. In one case, the extracorporeal

circulation was "turned off" for 45 minutes. The animals made satisfactory recoveries.

The brain, as might be expected, is markedly changed. It appears white and flattened. The vessels of moderate to minimal size are invisible on the surface. The larger vessels appear as fine lines, dark bluish-red in color. The brain substance seems toughened and there is a distinct difference in consistency between gray and white matter. It is relatively easy to distinguish vessels in the depth and these can be selectively occluded or visualized when required. Dissection is very easy. The brain separates along anatomical lines with great ease. A large frontal lobectomy extending posteriorly to the point behind the sphenoid wing required something less than 6 minutes for completion.

The systemic risks and disadvantages which are coincident with the desirable brain state seem to have been moderately well controlled. There was no cardiac fibrillation despite the relatively short cooling time and the low temperatures maintained after cooling. It is thought that fibrillation was avoided by blocking of the sympathetic system before cooling. Deep anesthesia also contributes to the avoidance of systemic complications.

It is hoped that this technique will provide a basis for clinical operative procedures and that its more fundamental development will provide information concerning the hibernation capabilities of higher primates. It seems clear that we can now suspend the greater metabolic activities of these chimpanzees for a period as long as 2 hours. Perhaps this period can be extended with greater refinement of the present technique. If so, the technique will not only provide a satisfactory basis for extensive intracranial operations on vascular malformations, it may also provide a reliable safeguard against the complications of intracranial hemorrhage and cerebral edema from other causes.

Significance to Neurological Research: This project studies the effects of low temperature on neurological activity. Its immediate and practical significance in neurological research is clinical. It is intended to provide a new baseline for neurosurgical technique. Under the conditions of deep hypothermia, the brain receives the maximum of protection during surgery and thereafter, while it also may be protected during hemorrhage and other intrusion occurring outside the operating room. This project, then, provides opportunity for therapeutic application in cerebral vascular disease, brain tumor, trauma, and abscess. In its fundamental and future applications, it may provide information concerning the capabilities of higher primates in the condition of low temperature. This suspended animation, if successful, may have some usefulness in further understanding of the thermoregulatory mechanism. Possibly it may have some application to the neurological aspects of space medicine.

Proposed Course of the Project: The project will be continued until the anesthetic, operative, and recording techniques are sufficiently predictable and accurate to justify its application in hospital cases.

Serial No. NINDB-66(c)
1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of Deep Hypothermia in Dogs

Principal Investigator: Anibal H. Galindo, M. D.

Other Investigators: Charles A. Bucknam, M. D.

Man Years (calendar year 1960)
Total: 4.0
Professional: 2.0
Other: 2.0

Project Description:

Objectives:

1. To study the effects of deep hypothermia on higher animals.
2. To develop a technique of deep hypothermia suitable for clinical application.

Methods Employed:

1. Extracorporeal cooling by femoral catheterization.
   a. DeWall 'bubble-oxygenator' with sigmamotor pump. This is used with 100% oxygen and flow of 20-40 cc/Kg/min.
   b. Temperature changes by a Harrison-Brown heat exchanger.
2. Recording of:
   a. arterial pressure
   b. venous pressure
   c. EKG lead II
   d. EEG (fronto-occipital)
   e. brain temperature
   f. esophageal temperature
   g. rectal temperature
   h. hematocrit
   i. blood pH
   j. plasma CO_2
3. Anesthesia with Thiopenthal; artificial respiration.
4. 50 dogs were used.

Major Findings:

1. Average cooling time 65 minutes.
2. Average warming time 75 minutes.
3. Mean low temperature:

a. Esophageal 4° C.
b. Rectal 7° C.
4. Average time "off pump" 1 hour.
5. Recovery rate 75%.
6. Sympathetic block protects against fibrillation.
7. Rate of perfusion is critical.

Significance to Neurological Research: The technique permits circulatory arrest for 60 minutes with recovery. Brain surgery is facilitated because of low intracranial pressure, altered consistency, and absence of bleeding. Thus it may provide a basis for more successful surgical treatment in cerebral vascular disease, arteriovenous malformations, aneurysms, and brain tumors.

Proposed Course of the Project: The technique will be applied to chimpanzees undergoing intracranial surgery. Thus it can be refined for clinical application. Fundamental studies of brain activity at low temperature will continue.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bucknam, C. & Galindo, A.: Tolerance of circulatory arrest in deep hypothermia by extracorporeal cooling. J. Neurosurg. (in press)

Bucknam, C. & Galindo, A.: Profound hypothermia by extracorporeal cooling. Surg. Forum, Vol. XI (in press)

Addendum to Serial No. NINDB-75(c) - 1959

1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Hypothermia in Neuroanesthesiology

Principal Investigator: William L. Pritchard, M. D.

Other Investigators: Charles A. Bucknam, M. D., Shelley Chou, M. D.

Man Years (calendar year 1960)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NINDB-75(c), 1959

Methods Employed: Same as NINDB-75(c), 1959

Major Findings: Same as NINDB-75(c), 1959

Proposed Course of the Project: This project, as outlined in NINDB-75(c), 1959, was discontinued upon termination of the appointment of the principal investigator.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Pritchard, W. L.: Selective head cooling in the dog. Neurology (in press)

Addendum to Serial No. NINDB-76(c) - 1959
1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Succinyl Choline in Awake Craniotomy

Principal Investigator: William L. Pritchard, M. D.

Other Investigators: Maitland Baldwin, M. D., Charles A. Bucknam, M. D.

Man Years (calendar year 1960)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NINDB-76(c), 1959

Methods Employed: Same as NINDB-76(c), 1959

Major Findings: Same as NINDB-76(c), 1959

Proposed Course of the Project: This project, as outlined in NINDB-76(c), 1959, was discontinued upon termination of the appointment of the principal investigator.

Part B included: No

Addendum to Serial No. NINDB-77(c) - 1959

1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Hypertonic Urea Solution on Intracranial Pressure

Principal Investigator: William L. Pritchard, M. D.

Other Investigators: None

Man Years (calendar year 1960)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NINDB-77(c), 1959

Methods Employed: Same as NINDB-77(c), 1959

Major Findings: Same as NINDB-77(c), 1959

Proposed Course of the Project: This project, as outlined in NINDB-77(c), 1959, was discontinued upon termination of the appointment of the principal investigator.

Part B included: No

Serial No. NINDB-67(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Psychological Evaluation of Temporal Lobe Operations.

Principal Investigator: Herbert Lansdell

Other Investigators: M. Baldwin, A. Mirsky, J. Caldwell, M. Mannarino S. Jones and N. Urbach.

Cooperating Unit: NIMH Section on Animal Behavior

Man Years (calendar year 1960):
Total: **4.0**
Professional: **1.0**
Other: **3.0**

Project Description:

Objective: To study patients with temporal lobe disorders with emphasis on the areas of intellectual ability, visual and auditory perception, linguistic functions and other more general "personality" features.

Methods Employed: Intelligence and personality tests; aphasia, audiometric, and other specialized verbal tests; tests of visual perception. Tachistoscopic recognition. Continuous performance test. Auditory testing during neurosurgery on conscious patients.

Major Findings:

1. The Wechsler-Bellevue test records in the files were rescored for all operated patients. There was no change in the Full Scale IQ from preoperative to postoperative in 44 cases of temporal lobe surgery, the average scores being 101. Forty-one follow-up cases also had the same average IQ of 101. The left temporal cases were slightly less intelligent than the right sided cases and their Verbal IQ tended to drop after the operation. These kind of phenomena have been reported in the literature by Milner for cases at the Montreal Neurological Institute; although we have a larger number of cases, the effect of the operations is not as clear statistically. The changes in the subtest scores of this test were similar to those reported by Meyer at Guy's-Maudsley Neurological Unit; the "Comprehension" score shows a drop. As with Meyer's study the change was not statistically significant. The comparison

of our scores for the left temporal group preoperatively with those of the left temporal follow-up group was significant at better than the 5 per cent level: there is a progressive drop in score from preoperative to postoperative and in the follow-up cases. However, our nontemporal cases show the same tendency on follow-up. A further statistical analysis of the relative proficiency on each subtest for each patient would now seem warranted.

In his latest revision of the intelligence test (which we do not use) Wechsler added a few proverbs to the Comprehension subtest. The pertinence of his addition may be seen in the results reported in the next section.

2. Gorham's Proverbs Test has proven to distinguish between the effects of left and right temporal surgery, but only in males (None of the right-sided cases made more errors after the operation, all of the left-sided cases have). There is also a marked difference in the mean scores of right and left sided follow-up cases (N=23). The nature of the immediate effect of the surgery in the female is obscure because of too few cases as yet; the scores of follow-up cases (N=13) differ, as with males, but not as strikingly.

3. The tendency reported last year with Bass' Famous Sayings has failed to achieve statistical significance with an adequate number of cases (N=17 temporal, 17 nontemporal, and 27 follow-up temporal cases).

4. Pettigrew's "Estimation Questionnaire" has provided unexpected results: all eight left-temporal cases increased the range of their rather low estimates, all six but one right cases reduced their higher estimates after operation; the follow-up group of cases (N=37) continued the direction of change with each mean score close to the deviate mean of the opposite group preoperatively. Seventeen other neurosurgical cases (pre- and post-operative) and seven follow-up cases had mean scores without deviation from "average." One might describe the results in terms of how the patients are "cognitively tuned" to their environment (Bruner); but at present it would seem advisable to put effort into checking the implication with some other relevant data on these patients--which have not been analyzed as yet.

5. The MMPI, a personality questionnaire, has shown a picture of improvement after surgery but of deterioration in the follow-up group. Attempts are being made to disentangle statistically the normal retest effect by comparison with other operated groups, and to provide some other corroboration for the reality of the generally unhealthy follow-up picture. Several other minor methodological problems have also been encountered in attempting to interpret the data (e.g., several of the follow-up group have high "lie" scores); and the large number of cases is not as encouraging as would be the case with simpler tests.

6. A word-association test with "correct" answers was invented and some normative data (N>300) obtained; the task is to choose the normal response to a "stimulus" word. A reliable correlation with the Wechsler IQ test was

obtained in a mixed patient group. The most relevant significant finding with temporal-lobe cases is a correlation in the follow-up group with the MMPI; those cases less able to pick the proper associate report themselves on the MMPI as less adjusted. This group's scores showed no correlation with intelligence, and other groups have failed to show a significant correlation with the MMPI. We are now exploring the possibility that as a test of "ability to appreciate common thought tendencies" it will provide part of the explanation needed for the results mentioned in Sections 1, 4 and 5 above. A preliminary report circulated among some specialists in word association research is eliciting some excellent advice.

7. A test of "closure" was used in the hope that it could discern some of the changes in visual perception that might be expected from temporal operations. This test requires the classification of pictures of human faces; each picture usually requires at least a moment before the stark highlights and shadows are resolved. Many right-temporal follow-up cases do rather poorly. The possibility of a selective aspect to such cases led to a more general consideration of the relation of this test to other variables such as age, IQ and MMPI scores; the interesting, but somewhat diverting, result was that anxiety (MMPI's "A" scale) was found to be positively related to the closure score in the females and negatively in the males (mixed group of patients). Some further analysis of this phenomenon in relation to our other questionnaires and perceptual tasks is needed before attempting to label the cases as merely deficient in visual closure ability.

8. Cattell's "16 Personality Factors" questionnaire has been used on a few cases. The form used was a modified one which includes all the items in his original three forms and the items are on cards to be sorted. One scale has produced results which may continue to describe changes after temporal lobe surgery. All six cases dropped on the "F" scale, while only one out of seven other neurosurgical cases did so. This tendency toward "Desurgency" is described by Cattell as being more "glum, sober, serious."

In summary, the nature of the psychological effects of temporal lobe surgery implied by these results would seem to require some additional statistical scrutiny, and more impressive control observations before insisting on gradually deleterious effects. (Of course, many of the patients derive obvious benefits with respect to their epilepsy, and in the consequent social adaptation.) But the contribution of temporal lobe function in normal human behavior may be more specifically inferred if the present data withstand our checking for extraneous factors. The temporal lobes would seem to be not especially involved in the types of tasks traditionally included in intelligence tests. Rather this part of the brain seems to be more involved in maintaining some balance in subtler functions tapped by verbal and perceptual tests where something is required that might be called the "judgment of the well adjusted person."

Significance of the program to the Institute: Several tests appear to distinguish the effects of temporal lobe surgery from other forms of neurosurgery and even the side of the surgery. There is evidence for some progressive change upon follow-up. Further analysis will probably provide some definition of temporal lobe function in normal emotional and intellectual function.

Proposed course of the project: Future effort will be in the further statistical analyses indicated by the present findings; several other tests not mentioned here (and designed to sharpen some of the distinctions found so far) will soon be yielding sufficient data for similar analyses.

Part B included Yes ☐ No ☒

Serial No. NINDB-68(c)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Observations on Vascular Permeability in the Brains of Rats Exposed to Alpha Particle Radiation.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: Jaime Miquel, Ph. D., C. Tobias, M. D. and Webb Haymaker, M. D.

Man Years (calendar year 1960):
Total: 1.5
Professional: .5
Other: 1.0

Project description:

Objective: This project was undertaken with collaboration of the Donner Laboratory, University of California and the Armed Forces Institute of Pathology and was supported by funds from the U. S. Atomic Energy Commission, Project # AT(49-7)-1646. The main objective was the assessment of vascular permeability changes in the brain produced by the alpha particle radiation.

Methods Employed: Brains of 63 rats were exposed to alpha particles at a surface dose of 6,000 rad from the 60-inch cyclotron in Berkeley, California. The cyclotron aperture through which the alpha particles passed was 14mm. in diameter, and thus was of sufficient size to allow irradiation of most of the dorsal surface of the cerebellum and cerebrum bilaterally. Following irradiation the rats were sacrificed at various time intervals. Prior to sacrifice the majority of the animals were injected intravenously with fluorescein labeled serum proteins (FLSP) others were injected with 10% sodium fluorescein. The alterations in the vascular permeability were assessed by gross and microscopic localization of the green fluorescence of FLSP

Methods Employed (con't):

and sodium fluorescein, by staining of erythrocytes with Pickworth Lepehne method, and by histological observations concerning the reaction of glia, neurons and other constituents of the brain parenchyma.

Major Findings: Our observations indicate that: (1) The primary noxious effect of radiation appears within 24 hours at the depth of the cerebral cortex and cerebellum in a horizontal band corresponding in intensity to the Bragg curve. This primary damage of the tissue within the Bragg zone is exemplified by the presence of pyknotic nuclear changes and appearance of PAS-positive droplets, scattered in the brain parenchyma. (2) The first vascular permeability changes appear at 48 hours after radiation and are demonstrable by microscopic leakage of FLSP from the capillaries, as well as by perivascular reaction of the astrocytes in the zone of irradiation damage. (3) Extravascular leakage of proteins can be demonstrated microscopically as late as 36 days after radiation with FLSP technique, whereas gross injury to the blood-brain-barrier, as tested with sodium fluorescein, ceases to be detectable after 18 days.

Proposed course of the project: This project is near completion and only requires some further analysis of the accumulated data.

Part B included: Yes [ ] No [X]

Serial No. NINDB-69(c)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study on Pinocytosis in the Nervous Tissue.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: Jaime Miquel, Ph. D.

Man Years (calendar year 1960):
Total: 1.5
Professional: .5
Other: 1.0

Project Description:

Objective: In view of the potential importance of pinocytosis for the uptake, transport and exchange of substances in the nervous system the present study was undertaken. It was our purpose to assess the pinocytotic activity of various nervous tissue cells grown in vitro as well as to search for evidence that pinocytosis may actually take place in the brain in vivo.

Methods Employed: Pinocytosis in vitro was studied: (1) by cinemicrographic time-lapse records in the phase contrast (2) by observations with fluorescence microscope on the characteristic pinocytotic uptake of fluorescein labeled proteins (FLP) from the medium. Pinocytosis in vivo was studied by uptake of FLP in form of characteristic inclusions in the glial cells seen in the area of edema in the white matter or in macrophages in the subarachnoidal space. In the latter experiments the cats were injected with FLP intravenously before a localized edema in the white matter was produced by application of the cold plate to the exposed surface of the cerebral cortex. Also, some animals were injected with FLP into the cisterna magna.

Major Findings: Observations in vitro demonstrated that the pinocytotic activity as seen with cinemicrography in the phase

Major Findings (con't'd):

contrast and the pinocytotic uptake of FLP were clearly correlated and characteristic for the individual cell type. The pinocytotic activity was most active in the microglial macrophages, but it was also present in astrocytes, oligodendrocytes and Schwann cells. It was absent in the nerve cells, on the other hand, it was vigorous in the perineuronal satellites. In vivo uptake of FLP by the glial cells in an area of edema and in the subarachnoidal space showed a striking resemblance to that observed in vitro, suggesting that the same pinocytotic mechanism is involved. Our observations with FLP in vivo emphasize the importance of glia as a metabolic intermediary and a transmitter of substances, and provide a technique for future investigations concerning the localization of serum proteins in the brain.

Proposed course of the project: This project is completed.

Part B included: Yes [X] No [ ]

Serial No. NINDB-69(c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Igor Klatzo, M. D. and Jaime Miquel, Ph. D.
"Observations on Pinocytosis in the Nervous Tissue".
Journal of Neuropathology and Experimental Neurology,
Vol. 19:475-487, October, 1960.

Honors and Awards relating to this project:

Serial No. NINDB-70(c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study on Morphological Changes due to Exposure to Radio-frequency Energy.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: M. Baldwin, M. D., S. A. Bach, M. D., E. J. Laskowski, M. D. , S. A. Lewis, R. N.

Man Years (calendar year 1960):
Total: 1.5
Professional: .5
Other: 1.0

Project Description:

Objective: This project is designed to provide the data on the morphological changes produced by exposure of primates to certain radio-frequency energy. It constitutes one of the aspects of the research Project # N-1-527 conducted in the Surgical Neurology Branch which is concerned with various problems connected with the effects of this type of energy.

Methods Employed: Primates are exposed to various ultra-high frequencies within micro-wave ranges as described in Project # N-1-527. Sodium fluorescein is used for study of the permeability of the blood-brain-barrier (BBB). Various histochemical and histological procedures are employed to assess the changes in the central nervous system of the primates which are sacrificed at various time intervals after exposures.

Major Findings: Preliminary findings indicate the following: in acute experiments, where the animals are sacrificed within a few minutes after exposure in the "low position" the changes in the permeability of the BBB can be found in the pons, medulla

Major Findings (cont'd):
and extending through the white matter into the cerebellar hemispheres. Sometimes, the BBB changes are localized lower and involve the dorsal areas of the cervical spinal cord. The histological changes in the acute experiments are confined to the striking tygrolysis in the large tegmental neurons of the pons, medulla and the dentate nucleus of the cerebellum. No neurofibrillary changes can be observed in these neurons.

In the chronic experiments only a few animals showed changes in the BBB and they were localized in the dorsal portions of the cervical cord. The morphological changes were found in the majority of the animals and were seen mainly in the dorsal areas of the cervical spinal cord. They consisted in severe tissue destruction, mainly in the posterior columns. In these areas small cystic cavities could be seen transversed by greatly proliferated blood vessels with abundant reticulin. Changes in the medulla and pons found in a few animals showed similar cystic cavities with well preserved neurons in the vicinity.

Proposed course of the project: This project has been temporarily suspended due to the work on instrumentation.

Part B included: Yes [ ] No [X]

Serial No. NINDB-71(c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of Vascular Permeability in the Central Nervous System using Fluorescein Labeled Serum Proteins (FLSP) Technique.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: Jaime Miquel, Ph. D.

Man Years (calendar year 1960):
Total: 1.5
Professional: .5
Other: 1.0

Project Description:

Objective: In the course of our study on pinocytosis in the nervous tissue (Project No. NINDB 60 (C) -1959) a technique has been developed which allows the microscopic localization of fluorescein labeled serum proteins (FLSP) in the brain tissue sections. Thus, any abnormal, extravascular passage of FLSP into the brain parenchyma, as well as, cellular uptake and transport of these proteins can be followed under high optical resolution using a fluorescence microscope. The main objective in this project is to observe microscopically alterations of vascular permeability and of various brain "barriers" under different experimental conditions and also to assess the role and the participation of glia with regard to the intraparenchymal transport of extravasated serum proteins.

Methods Employed: Cats and monkeys were injected intravenously with FLSP (mainly albumin) and following the injection a localized edema in the white matter was produced by application of a cooled to -50° C metal plate to the exposed cerebral cortex. Some animals were injected with FLSP intraventricularly or intrathecally. The animals were sacrificed at various time intervals. The brain tissue

Methods Employed (Cont'd):

blocks were fixed in 10% formolsaline and 15 μ thick sections were cut on the freezing microtome. The sections were studied and photographed under the fluorescence microscope.

Major Findings: In the area of edema in the white matter an irregular, "mottled" distribution of FLSP was observed within the first hours after the cold injury. Later, this "mottled" brightly green fluorescence tended to disappear revealing neuroglial cells showing distinct FLSP intracytoplasmic inclusions. These inclusions are strikingly similar to the pinocytotic inclusions observed in the glial cells grown *in vitro* and thus the pinocytotic origin is attributed also for the glial inclusions *in vivo*. After 48 hours two distinct routes of the FLSP transport were recognizable; one towards the pia and the other towards ependyma. The microglial cells appeared to be mainly involved in this transport. Following the intraventricular or intrathecal injection of FLSP no intraparenchymal invasion was observed in animals which did not show any signs of unduly increased intracranial pressure. In animals which tolerated poorly these injections and developed an acute increase of the intracranial pressure a diffuse green fluorescence was observed in the superficial band of brain tissue facing the pial and ependymal surfaces.

Proposed course of the project: This project is near completion. For further elucidation of the findings it requires more data from the larger groups of animals.

Part B included: Yes ☐ No ☒

Serial No. NINDB-72(c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Histochemical Study on Central Core Disease

PrincipalInvestigator: Professor Franz Seitelberger

Other Investigators:

Man Years (calendar year 1960):
Total: .5
Professional: .5
Other: 0

Project Description:

Objective: To determine by histochemical methods the reactivity of the central core portion as compared to the peripheral part of the muscle fiber and thus to gain insight into the pathogenic mechanisms involved in this disease.

Methods Employed: Muscle biopsy material was obtained from a member of the family in which this clinico-pathological syndrome was first described (Shy and Magee, 1956). Orientated pieces of muscle were fixed in formalin-saline, Bouin, 10% neutral formalin and alcohol-formalin in a ratio of 9:1. For histologic examination paraffin sections were cut from formalin-saline and Bouin fixed blocks. They were stained with H and E, Gomori trichrome and van Gieson. For histochemical purposes frozen sections were prepared from formalin fixed material and subjected to the following reactions: PAS for carbohydrates; Sudan black for fats and lipids; Amido-black IOB for protein; cresyl violet for basophilia; and Alcian Blue for acid-mucopolysaccharides. A group of sections was treated with fat solvents such as pyridine, ether, chloroform-methanol (1:1), 70% ethyl alcohol, and 96% ethyl alcohol; these were then, together with untreated control sections, stained with PAS. Paraffin sections were prepared from alcohol-formalin fixed and formalin fixed material, stained with the aforementioned methods, and in addition, treated with Best's carmine before and after

Methods Employed (cont'd):

diastase treatment. They were also tested for acid mucopolysaccharides by their capacity to combine with iron. The isoelectric point was determined on formalin-fixed sections by means of methylene blue extinction. The presence of amyloid was tested with crystal violet.

Major Findings: The histochemical determinations indicate that there is less glycogen in the core area as compared with the periphery. A similar relationship is found with regard to structures containing lipids, basophilic material and acid mucopolysaccharides. The isoelectric point is at pH 4.33. There are no definite indications of chemical changes in myofibrillar structures although a structural mucopolysaccharide fraction appears more concentrated in the core area.

These findings show that reactions which indicate the presence of structures in the interfibrillar space are negative in the core area. On the other hand, there is an increase of fibrillar material in the core region.

Significance to Program of Institute: The data obtained indicate that the changes in Central Core disease are not qualitative in nature but rather seem to consist of an aberrant distribution of physiological material present in the normal muscle cell.

Since, however, dystropic formations frequently are combined with structural defects, it is possible that such changes also exist in this disease; they may escape detection because of the limitations of the methods employed.

Proposed Course of this Project: The results of this investigation will be correlated with histological and electron microscopic findings on this disease. The integration of the data obtained by these different disciplines may yield more information on the structural changes occurring in this syndrome.

Part B Included Yes [ ] No [X]

Serial No. NINDB-73(c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Observations on Alterations in the Vascular Permeability in the Experimental Allergic Encephalitis.

Principal Investigator: Prof. Dr. F. Seitelberger

Other Investigators: Igor Klatzo, M. D. and Jaime Miquel, Ph. D.

Man Years (calendar year 1960):
Total: .5
Professional: .5
Other: 0

Project Description:

Objective: The exact mechanism and nature of the experimental allergic encephalitis (EAE) is still unknown. Alterations in vascular permeability are assumed to play an important part in the pathogenesis of this condition. Our objective was to study these alterations with the sensitive fluorescein labeled serum protein (FLSP) technique (described in Project 1960).

Methods Employed: Ten monkeys were injected with guinea-pig brain homogenate mixed with Freund adjuvant. The animals received 3 injections each at weekly intervals. Seven animals developed typical signs and symptoms, usually within one week after the last injection. Some of these animals were sacrificed when the first symptoms appeared, the others after several days of sickness. Prior to the sacrifice the animals received intravenous injection of the FLSP. The tissue was fixed in 10% formalin and sectioned on the freezing microtome for fluorescence study. In addition, the corresponding sections were stained with routine histological methods.

Major Findings: The fluorescence observations concerning the localization of FLSP revealed the following: (1) In animals sacrificed after the EAE symptoms appeared there could be seen

Major Findings (cont'd):

some arteries which showed green-fluorescent perivascular exudates of FLSP without the presence of inflammatory cells in this location. These exudates were confined to the Virchow-Robin spaces. (2) There was present a considerable number of veins surrounded by dense cuffings of inflammatory cells without associated evidence of increased permeability of FLSP. (3) Some veins with inflammatory cells also showed FLSP within the Virchow-Robin space. (4) Occasionally FLSP exudates could be observed within the stroma of the choroid plexus. (5) In some areas there was wide parenchymatous FLSP spreading, similar to the "mottled" appearance described in experimental edema (K&M). These areas were usually observed in the white matter and occasionally were devoid of any inflammatory cells. The relationship of these exudates to the blood vessels was difficult to determine since they showed a tendency to irregular coalescence. (6) In animals sacrificed at a more pronounced stage of the disease some obviously older lesions, associated with perivascular neutral fat deposits, demyelination and intense astrocytic proliferation did not reveal any associated increased permeability of FLSP. (7) In one animal which was sacrificed in a moribund state in addition to extensive areas of increased FLSP permeability in the brain parenchyma and in the choroid plexus there was green fluorescence staining of the cytoplasm of the subpial astrocytes. This astrocytic appearance presumably represents uptake of FLSP by these astrocytes from the subarachnoidal space. (8) The animals sensitized with brain plus Freund adjuvant which showed no clinical symptoms of the disease revealed no histological lesions nor increased permeability of FLSP.

Proposed course of the project: A second series of animals will be required to complete this project. In particular, of great interest should be examination of animals which survived acute phase of EAE and could be sacrificed at the later stages.

Part B included: Yes [ ] No [X]

Addemdum to Serial No. NINDB-62(c) - 1959
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Histochemical and Electrophysiological Observations on the Muscle Fibers Grown in Vitro.

Principal Investigator: W. K. Engel, M. D. (Medical Neurology)

Other Investigators: Choh Lu Li, M.D. and Igor Klatzo, M.D.

Man Years (calendar year 1960):
Total: 0
Professional: 0
Other: 0

Project Description:

Objective: Same as NINDB 62 (C) - 1959

Methods Employed: Same as NINDB 62 (C) - 1959

Major Findings: Same as NINDB 62 (C) - 1959

Proposed course of the project: Certain aspects of this project are completed, and are being continued by the Principal Investigator who is now with the Medical Neurology Branch.

Part B included: Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Engel, W. K. : Cytological Localization of Glycogen in Cultured Skeletal Muscle.

Engel, W. K. : Cytological Localization in Cholinesterase in Cultured Skeletal Muscle Cells.

Both articles appearing in:

The Journal of Histochemistry and Cytochemistry,
Vol. 8, November, 1960.

Honors and Awards relating to this project:

Addendum to NINDB 63 (C) 1959
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: A New Method for Quantitative Study of Precipitin Reaction.

Principal Investigator: Jaime Miquel, Ph. D.

Other Investigators: B. Horvath, M. D. and Igor Klatzo, M. D.

Part B. Honors, Awards and Publications

Publication other than abstracts from this project:

Miquel, J., Ph. D. Horvath, B., M. D. and Igor Klatzo, M. D. "A Chromatographic Technique for the Quantitative Study of the Precipitin Reaction", The Journal of Immunology, Vol. 84, No. 5, May 1960, 545-550

Honors and Awards relating to this project:

Addendum to Serial No. **NINDB-64(c) - 1959**

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of the effects of Hypothermia on Injured and Normal Brain Tissue.

Principal Investigator: Edward J. Laskowski, M. D.

Other Investigators: Igor Klatzo, M. D.

Man Years (Calendar year 1960):
Total: 0
Professional: 0
Other: 0

Project Description:

Objective: Same as NINDB 64 (C) - 1959

Methods Employed: Same as NINDB 64 (C) - 1959

Major Findings: Same as NINDB 64 (C) - 1959

Proposed course of the project: This project is completed.

Part B included: Yes [X] No [ ]

Serial No. NINDB-64(c) - 1959

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Laskowski, E.J. : Observations on the Effect of Hypothermia on Experimental Brain Lesions. The American College of Surgeons, "Surgical Forum". Vol. IX,: 714-717, 1959.

Laskowski, E. J., Klatzo, I., and Baldwin, M.,: Experimental Study of the Effects of Hypothermia on Local Brain Injury. Neurology, Vol. 10, No. 5, May 1960, 499-505.

Honors and Awards relating to this project:

Addendum to NINDB 75 - 1958
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Localization of Myosin in Human Striated Muscle by Fluorescent Antibody.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: B. Horvath, M. D. and W. K. Engel, M. D.

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Klatzo, Igor, M. D., Horvath, Beni, M. D. and Engel, W. K., M. D.
"Observations on Myosin in the Abnormal Muscle using Fluorescent Antibody Technique".

Published in the Association for Research in Nervous and Mental Diseases. Volume 1960.

Honors and Awards Relating to this project:

Addendum to NINDB 76 (C) - 1958
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of Pathology of Kuru Disease.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: D. C. Gajdusek, M. D. and V. Zigas, M. D.

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Klatzo, I., Gajdusek, D. C. and Zigas, V. : "Evaluation of Pathological Findings in Twelve Cases of Kuru".

Published in the Proceedings of the International Symposium of Actual Encephalitides, Antwerp, Belgium. 1960

Honors and Awards relating to this project:

Serial No. NINDB-74(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigation of the Etiology, Pathology and Clinical Manifestations of Cerebral Palsy and Allied Conditions; Also of the Epilepsy During Childhood.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: .8
Professional: .2
Other: .6

Project Description:

Objectives: Comprehensive clinical, laboratory and genetical investigation of a selected group of children suffering from organic brain syndromes or epilepsy and correlation of thus obtained data with the findings in pneumoencephalogram. The main objectives are: 1 - Correlation of the clinical features of cerebral dysfunction with the site, size and character of the cerebral lesion. 2 - Classification of a larger group of children suffering from organic brain condition according to the etiological factors whenever these can be established. 3 - Preparation of publications based on smaller groups of patients presenting particularly important aspects in relation to pathology, pathogenesis or response to a new type of treatment.

Methods Employed:

1. Genetic investigation

2. Detailed neurological examination, including developmental testing and electroencephalographic studies.

3. Pneumoencephalogram.

4. Biochemical assays.

5. Other special tests as indicated.

Patient Material: In-patients

Clinical Project

Major Findings:

During 1960, 40 patients were studied in great detail as in-patients and 32 on the out-patient basis. In addition 28 neurological consultations were rendered.

Material from a considerable proportion of the in-patients formed the basis of communications already published as listed below and the part of the material is currently evaluated. Our main purpose is to document hitherto unknown factors responsible for congenital malformations or brain damage - as it was done in our studies on offspring of diabetic mothers - or to contribute to clarification of clinical and pathological aspects of cerebral palsy, epilepsy and mental deficiency.

An example of the value of such approach can be seen in the analysis of six selected infants suffering from frequent daily epileptic attacks. These patients were studied in great detail clinically, electrographically and by means of biochemical tests. Also, they all had pneumoencephalograms done. On the basis of complete appraisal of their neurological status it was felt that the cerebral seizures in this instance were of idiopathic type not associated with organic brain damage. The important aspect of this study is the finding that these children began to slow in development concurrently with daily increase in seizures and their mental state was improving when the attacks were controlled. This impression was also confirmed indirectly on longer range longitudinal study. As a result of these investigations we were able to put forward the following hypotheses:

1. Epilepsy of idiopathic type can occur in infants under 1 year of age.

2. Frequent daily attacks of "petit mal" and "petit

mal variant" occurring under 1 year of age can by themselves cause significant and irreversible mental retardation.

During subsequent years efforts will be made to test validity and limitations of these hypotheses.

Results of studies on a number of patients are reported in the publications listed below. Final analysis of the material has to await accumulation of more patients.

Significance to Neurological Research: In a majority of cases the etiology of mental deficiency, cerebral palsy and allied conditions is poorly understood. Better knowledge of hereditary factors, clinical manifestations, as well as the location and extent of the lesion may further our insight into the diverse etiology of these conditions. Full understanding of pathology and etiology in larger groups of children with brain damage will suggest eventually better directed preventive and therapeutic measures.

Proposed Course of the Project: This project has a wide scope and constitutes major interest of the Section, it, therefore, can be considered as a permanent, standing project.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Dekaban, Anatole: Idiopathic Epilepsy in Early Infancy. The question of frequent daily attacks causing undifferentiated type of mental deficiency. Am. J. of Dis. of Children 100: 181-188, 1960.

Dekaban, Anatole: Cerebral Birth Injury. Pathology of hemorrhagic lesions. Trans. A.R.N.M. Diseases. In press.

Honors and Awards Relating to this Project:

1. Assistant Professor of Neurology at George Washington University Medical School.

2. Consultant District of Columbia Children's Hospital.

Serial No. NINDB-75(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Maternal Condition During Pregnancy and the Course of Birth in Relation to Neurological Abnormalities in the Infants and Pathologic Lesions in Products of Abortion.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: Dr. T.E. Cone and Dr. H.H. Hill, National Naval Medical Center; Dr. L.J. Geppert and Dr. H.L. Riva, Walter Reed Army Hospital

Cooperating Units: National Naval Medical Center and Walter Reed Army Hospital

Man Years (calendar year 1960):
Total: .80
Professional: .20
Other: .60

Project Description:

Objectives: Analysis of various abnormal factors occurring in pregnant mothers or complications of birth which may cause or contribute to neurological abnormalities in infants.

Methods Employed:

1. Prenatal care of mothers under research and their individual final assessment.

2. Recording and evaluating of the course of birth and pertinent abnormalities.

3. Examination of the newborn infants during initial hospital stay.

4. Follow-up examination of infants

5. Gross and microscopic examination of the products of abortion and deaths.

Patient Material:

1. All pregnant women who were receiving prenatal care at the National Naval Medical Center and Walter Reed Army Hospital and subsequently were delivered in these hospitals between March 1, 1956 and March 1, 1957.

Clinical Project

Major Findings: The total of 4,156 pregnancies and products of these gestations were studied in great detail regarding prenatal care, confinement, infant's state at birth and his subsequent course up to one year of age.

The following tables give some insight into the extent of the material processed and our major findings:

TABLE I

Maternal Age by Group and Percent Distribution Within Each Group

Maternal Age Years	Abnormal Group A	Normal Control Group B	Abortions Group C	Percent Distribution		
				Abnormal	Normal	Abortion
Under 18	54	26	12	4.2	3.6	3.8
Between 18 & 28	820	516	155	64.3	70.8	49.7
Between 29 & 38	368	175	122	28.8	24.0	39.1
Over 38	35	12	23	2.7	1.6	7.4
All Ages	1277	729	312	100.0	100.0	100.0

TABLE II

Positive and Negative Family History for Likely Hereditary Disorders by Group and Percent Distribution Within Each Group.

Family History for Likely Hereditary Disorders	Abnormal Group A	Normal Control Group B	Abortion Group C	Percent Distribution		
				Abnormal	Control	Abortion
Positive	95	40	7	7.4	5.5	2.2
Negative	1073	636	148	83.5	87.0	47.1
Not Recorded	117	55	159	9.1	7.5	50.7
Total	1285	731	314	100.0	100.0	100.0

TABLE III

Past Abortions and Percent by Group and Number of Abortions

Past Abortions (From History)	Abnormal Group A	Percent	Control Group B	Percent
One Abortion	218	17.1	67	9.2
Two Abortions	53	4.2	17	2.3
Three or More Abortions	32	2.5	12	1.6
Abortions of Twins	2	0.2	-	-
Not Recorded	6	0.5	6	0.8

TABLE IV

Abnormalities of Surviving Offspring by Group

	TYPE OF ABNORMALITY	GROUP A Complicated	GROUP B Controls	TOTAL
Abnormalities, Severe 28 Instances	Hydrocephalus	1	1	2
	Congenital Malformations, Disabling	10	-	10
	Congenital Heart Disease	12	4	16
Abnormalities, Moderate 65 Instances	Convulsions	20	6	26
	Febrile	(12)	(3)	
	Afebrile	(5)	(2)	
	In organic brain les.	(3)	(1)	
	Retardation of Develop.	30	9	39
	Less than 20% of Norm.	(15)	(6)	
	21-35% of Normal	(10)	(1)	
	Over 35% of Normal	(5)	(2)	
Abnormalities, Mild 96 Instances	7th Nerve Palsy (Birth Injury	5	-	5
	Marked Squint	3	2	5
	Pyloric Stenosis	2	2	4
	Hernia	30	5	35
	Umbilical	(25)	(4)	
	Inguinal	(5)	(1)	
	Joint and Ligamentous Abnormalities	27	8	35
	Hemangioma, Skin	9	3	12
Sum				

Table IV shows a very important finding in our study namely the type and distribution of abnormalities present in 189 offspring of the studied series. Congenital malformations, retardation of development and epileptic attacks constituted the most serious abnormalities encountered.

As evident in the findings of this study, malformation was the cause of 18 fetal, neonatal or infantile deaths and also the cause of a serious handicap in 26 surviving offspring. This finding is a good example of a general rule that the same abnormality - or factor causing it - may be lethal when severe, sublethal when pronounced and handicapping when of moderate degree. Consequently, the

study of fetal and infant mortality is of considerable importance in appraisal of the causes and pathology of clinical abnormalities in the surviving offspring.

Significance to Neurological Research: It is postulated that various environmental factors acting during prenatal, intranatal and early postnatal life may be responsible for brain damage and the corresponding clinical sequelae in infants. This study may reveal the relative importance of certain factors related to gestation and parturition and also their incidence. Since careful and uniform examinations are being conducted during all stages of prenatal and postnatal life, final analysis of the findings should be significant.

Proposed Course of the Project: Currently two papers pertaining to the evaluation of the data are ready for publication. Much more work and efforts will be dedicated to analysis and preparation of various parts of this extensive material.

Part B included Yes / / No /x/

Serial No. NINDB-76(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Pathological Lesions in the Central Nervous System Occurring During Prenatal, Intranatal and Early Postnatal Life.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: Joan Crowe

Cooperating Units: None

Man Years (calendar year 1960):
Total: .8
Professional: .2
Other: .6

Project Description:

Objectives: The causation and pathology of the majority of patients with mental deficiency and cerebral palsy are largely unknown. Detailed examination of the brains of children who suffered from such disorders and correlation of these findings with the clinical data is expected to provide valuable information for elucidation of etiology of such conditions and eventually they may suggest possible preventive measures.

Methods Employed: Comprehensive examination of brains and spinal cords from patients who suffered from cerebral palsy or allied conditions by means of:

1. Detailed description of gross examination and dissections.

2. Microscopical study of large sections which were treated with chromatic, silver, myelin and fat stains as well as by various histochemical procedures.

Material:

1. Fifteen brains of infants who died in acute phase following cerebral birth injury.

2. Eight brains of children with the diagnosis of mental deficiency or cerebral palsy in chronic phase are currently being processed histologically.

Neuropathological Project

Major Findings: Fifteen brains of infants who died at the age of less than 3 days following cerebral birth injury were studied in great detail using modern neuropathological techniques. In the first phase of investigation the attention was focused on hemorrhagic lesion in the brain. The following two tables summarize pertinent clinical data coincidental to the birth injury.

In general, six main types of hemorrhagic lesions in the brain which resulted from birth injury were distinguished:

1. Hemorrhage associated with tear of the tentorium or cerebral falx.

2. Massive, focal subarachnoid hemorrhage associated with hemorrhagic lesions in the brain parenchyma.

3. Intraventricular hemorrhage.

4. Hemorrhagic infarction.

5. Direct, traumatic bruising of a part of the brain.

6. Minor, extravasations of blood in the subarachnoid space and isolated petechiae in the brain.

An attempt was made to correlate the type of the lesion with the mechanism of its production and with the corresponding counterpart of such lesions in chronic phase and in still surviving patients. The following is an example of this approach:

TABLE I

Birth Weight (Weeks Gestation)	Minor Subarach. Hem.	Focal, Massive Subarach. Hem.	Intra-Ventr. Hem.	Tentori-al Tear	Total
2500 GM.+ (39.40 W.G.)	1	6		1	8
1500-2500 GM (36-38 W.G.)			3		3
900-1500 Gm. (32-36 W.G.)		2	2		4
Total	1	8	5	1	15

TABLE II

Type of Delivery	Minor Subarach. Hem.	Focal, Massive Subarach. Hem.	Intra-Ventr. Hem.	Tentori-al Tear	Total
Apparently Normal, Spontaneous		1	1		2
Spontaneous Prolonged & Difficult		3	2		5
Breech Extraction	1	1	1		3
Midforceps		1			1
Low Forceps Difficult		1	1	1	3
Low Cervical Section and Forceps Extr.		1			1
Total	1	8	5	1	15

The fourth type of cerebral lesion studies is hemorrhagic infarction. One of the infants died 24 hours after difficult birth which necessitated external version

from transverse position and midforceps extraction. The infant's occiput at birth was greatly elongated. Examination of his brain revealed hemorrhagic infarction of both occipital lobes with normal remaining portion of the brain. The acute lesion of this brain was compared with sclerotic ulegyria affecting exclusively occipital lobes in a cerebral palsy patient who died at 20 years of age. He had a well documented history of traumatic birth and had cortical blindness and epilepsy. A still surviving 8 year old child who also has cortical blindness and epilepsy following traumatic birth was shown to have enormous dilatation of both posterior horns whereas the remainder of the lateral ventricles was normal - the finding consistant with destruction of both occipital lobes. The analysis of the data in these three categories of patients leads to a formulation of a plausible hypothesis that rapid extraction of the fetus or prolonged difficult birth in certain positions causes great elongation of the occiput with consequent stretching and compression of posterior cerebral arteries causing infarction of the occipital lobes. In the past, no good clues were available to interpret the pathogenesis of symmetrical occipital ulegyria. The results in more detail were published (see project # NINDB-74(c)) in the Trans. A.R.N.M. Diseases. In press.

Significance to Neurological Research: Such studies are of great importance as the number of brains examined in detail in acute phase of birth injury as well as of cerebral palsy and allied conditions is small.

Part B included Yes / / No /x/

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Dekaban, Anatole and Bartelmez, George: The early development of the human brain. Contributions to Embryology. In Press.

Honors and Awards Relating to this Project: None

Serial No. NINDB-77(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Incidence and the Type of the Central Nervous System Abnormalities Encountered in Offspring Born to Diabetic Mothers.

Principal Investigators: Anatole S. Dekaban, M.D.
Robert L. Baird, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Our clinical and neuropathological studies indicated that severe abnormalities must occur not infrequently in infants born to diabetic mothers. (Dekaban, A. and Magee, K.: Occurrence of neurological abnormalities in infants born from diabetic mothers. Neurology 8: 193-200, 1958). It became important to evaluate statistically the incidence of these abnormalities in larger series of offspring born to diabetic mothers and to analyze the findings in light of findings in series of normal controls.

Methods Employed:

1. Critical assessment of maternal diabetes and her total pregnancies.

2. Examination of her offspring.

Material: The outcome of 234 pregnancies in 48 diabetic women and in 249 pregnancies in 48 normal controls were analyzed. The mothers were personally interviewed and the offspring examined.

Clinical project

Major Findings: The outcome of 235 pregnancies in diabetic and prediabetic women were investigated and the results obtained were compared with the corresponding data in the 249 pregnancies of matched normal controls. The percentage of abortions and previable deaths in the diabetic sample was 29.9, in the prediabetic sample 20.5 and in the normal controls 12.4. Stillbirths occurred with the following frequency: 11.5 per cent in the sample of diabetic mothers, 5.1 per cent in the prediabetics and 1.2 per cent in the normal controls. The neonatal deaths amounted to 8.3 per cent of all pregnancies in the diabetic women, 1.3 per cent in the prediabetics and 3.6 per cent in the sample of normal controls. These values demonstrate clearly that the fetal wastage in the diabetic women is significantly greater than in the normal controls. The fetal loss during the prediabetic stage falls about midway between that in diabetic and normal control mothers.

The main purpose of our study was to test the hypothesis that the morbidity dating since birth in the offspring of diabetic mothers is also increased as compared with the sample of normal controls. There were six abnormal surviving offspring in the sample of 157 diabetic pregnancies which amounts to a total of 3.8 per cent (or 7.6 per cent of the surviving children). In the normal control sample of 249 pregnancies there was only one abnormal surviving offspring which constitutes the incidence of 0.4 per cent. The difference in percentages in these two groups was significant at P less than 0.01 indicating that our hypothesis is very likely correct. The abnormalities in these children include mental deficiency, congenital malformations, birth injury and epilepsy.

Three of the six abnormal surviving children born to diabetic mothers had severe congenital malformations, two had mental deficiency and one sequelae of birth injury and epilepsy. Of the forty-one offspring of diabetic women who were stillborn or died during the neonatal stage sixteen had comprehensive postmortem examination and the major findings are as follows: pulmonary hyaline membrane disease was found in eight patients, congenital malformations in three, cerebral birth injury in two and no gross, demonstrable lesion

in the remaining three. Factors, possibly pertaining to the increased fetal loss and to the higher rate of morbidity among the offspring of diabetic mothers are discussed in publications listed below.

Significance to Neurological Research: The four publications which are the result of our studies established for the first time that maternal diabetes should also be included to the known causes of mental deficiency and cerebral palsy in a proportion of offspring.

Proposed Course of the Project: This project has been completed. Two publications resulted: (1) Dekaban, Anatole and Baird, Robert: The outcome of pregnancy in diabetic women. Part I. Fetal wastage, mortality and morbidity in the offspring of diabetic and normal control mothers. J. Pediat. 55: 563-576, 1959. (2) Dekaban, Anatole: The outcome of pregnancy in diabetic women. Part II. Analysis of clinical abnormalities and pathological lesions in offspring of diabetic mothers. J. Pediat. 55: 767-776, 1959.

Part B included Yes [ ] No [x]

Serial No. NINDB-78(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Measurements of External and Internal Orbital Distance in Males and Females from Birth to Adulthood.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: It has been found that the measurement of the interpupillary distance in humans for the purpose of estimation of hypertelorism and abnormality of the sphenoid bone is unsatisfactory. It is thought that either external or internal orbital distance or index thereof should take place of the measurements of the interpupillary distance.

Methods Employed:

1. Measurements of the above-named distances in human males and females at progressive ages beginning from zero to 20 years of age.

2. Correlation of physical measurements of a small group of children with measurements made on cephalometric x-rays.

3. Statistical analysis in various age horizons.

Material: Measurements of all horizons have been taken. This amounts to the total of 600 head measurements.

Clinical Project

Major Findings: Part of the data derived from this study was analyzed and published in the book "Neurology of Infancy", pages 35-37. Entirely new are data pertaining to the norm in various ages of the interorbital distance and also to the volume of the skull as measured in live subjects.

Significance to Neurological Research: To make the estimation of conditions such as hypertelorism more scientific, the measurements of stable bony structures rather than movable organ as eyeball, should be performed. As an example a concommitant divergent strabismus can be given; in this instance measurement of the interpupillary distance for the estimation of the abnormality of the sphenoid bone would obviously give false results.

Proposed Course of the Project: The material is in the process of evaluation and preparation for publication.

Part B included Yes [ ] No [x]

Serial No. NINDB-79(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Preparation of the Horizon of the Normal Development of the CNS in Mice and Experimental Production of Congenital Malformations of the CNS.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: Marie J. Kendall, M.S.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.1
Professional: .1
Other: 1.0

Project Description:

Objectives: The purpose of this project is the production and analysis of congenital abnormalities of the CNS and the provision of norms for the development of the CNS in mice.

Methods Employed:

1. Dissection of the CNS of mice of 16 progressive developmental stages.

2. Preparation and staining of serial sections.

3. Identification and outlining of main structures on the low power microphotographs.

4. X-ray radiation of pregnant mice from a strain which does not show any significant incidence of spontaneously occurring abnormality of the CNS.

5. X-ray radiation of pregnant Black C 57 mice in several stages of pregnancy with similar parameters to these for mice in Swiss Albino; the strain used here show an abnormally high incidence of spontaneous malformations of the CNS.

6. Gross, skeletal, and microscopical examination of the obtained specimen.

Material: Mice strain: NIH stock "general purpose Swiss Albino" and Black C 57.

Major Findings: An atlas of normal mouse brain has been prepared and bound. It is in current use in our laboratory.

Careful dissection of brain and brainstem of fetus and young mice in eleven age horizons were performed. The specimens are sectioned serially and stained. It needs to be stressed that to obtain one perfect set of serial sections for one horizon it is usually necessary to process and section six to twelve brains. Only those sets which are in ideal conditions can be utilized for description.

Production of malformations by means of x-radiation. We are considerably limited in space for maintenance of mice. Since only certain age mice can be used, we have to harbour them until they attain it. Then, only about 20 percent of those kept become pregnant as a result of restricted duration of mating time. In strain "general purpose Swiss Albino" 98 litters were obtained from irradiated mothers. Approximately 10 percent of these had major abnormalities, about 25 percent minor abnormalities and the remaining are free of detectable pathology. Similar parameters of irradiation and technique were applied to strain Black C 57 and so far 45 litters were obtained.

Significance to Neurological Research: An experimental approach to congenital malformations of the CNS is necessary to help us understand certain obscure malformations occurring in humans. The provision of norms of the central nervous system has to precede the experimental production of congenital malformations, as there does not exist any proper guide in the form of an atlas or of a satisfactory reference during consecutive stages of the development of the mouse. Majority of the stages in this strain are not yet completed and final analysis of data and comparison with the findings learned from irradiation will be done during the coming year.

Proposed Course of the Project: For technical reasons we had to stop further irradiation of mice for the time being. It is planned to resume this experimental work as soon as our laboratory's output will increase to permit processing of additional material.

Part B included Yes / / No /x/

Serial No. NINDB-80(c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of Chromosomes in Patients with Congenital Malformation and in Mongoloids.

Principal Investigator: Anatole Dekaban, M. D.
Michael Bender, Ph.D. (Oak Ridge Laboratory)

Other Investigators: Miss Peggy Barbee

Cooperating Units: Biological Laboratory, AEC, Oak Ridge, Tennessee (Dr. M. Bender)

Man Years (calendar year 1960):
Total: .7
Professional: .1
Other: .6

Project Description:

Objectives: Recently it has been found that a proportion of patients with profound congenital malformations and also mongoloids have abnormal chromosomal constitution. Since chromosomes contain hereditary material responsible for expression of all traits - the abnormality in the number and in patterns may be associated with or even be a cause of malformation. This approach is likely to open a new field in research on congenital malformations.

Methods Employed:

1. Comprehensive clinical and laboratory investigation of selected patients to establish diagnosis and degree of abnormality.

2. Tissue culture of the peripheral blood after red blood cells are removed. Bacto-Phytohemagglutinin is added in order to stimulate proliferation of the white blood cells. 6 hours prior to sacrificing the culture colchicine is added in a final concentration of 0.5 x 10-6 M.

3. Through a number of steps the white blood cell suspension is centrifuged and washed, then exposed to hypotonic salt solution and fixed.

4. Concentrated suspension of cells are stained on the slide with orcein, Giemsa or Feulgen methods.

5. Under high power resolution in light and contrast-phase microscope the chromosomes are counted, their morphology studied and caryotype figures made for final analysis.

Material: Patients with congenital malformation, epilepsy and mental deficiency.

Major Findings: This is a new project.

Significance to Neurological Research: The etiology and pathogenesis of vast number of conditions associated with congenital malformation and mental deficiency are largely unknown. Demonstration of chromosomal aberration in some of these patients will be a great step forward in our understanding of these conditions and it may even suggest in a distant future certain preventive measures.

Proposed Course of the Project: This is likely to be a long term project.

Part B included Yes [ ] No [x]

SUMMARY OF ANNUAL REPORTS (1960)

Sanford L. Palay

The Laboratory of Neuroanatomical Sciences was founded in 1954 when Dr. William F. Windle was appointed chief. He rapidly collected a group of outstanding scientists to conduct the investigative work of the Laboratory, and although it was hampered at first by the turmoil of repeated moves from one building to another and by reconstruction of the quarters in its definitive building, the staff has initiated and carried forward research in all of the aspects of anatomical science at an increasing pace. Under Dr. Windle's leadership the laboratory has grown not only in size but also in stature so that it is now one of the principal neuroanatomical laboratories in the world. During the current year Dr. Windle resigned his position as Chief of the Laboratory in order to accept new responsibilities as Assistant Director of NINDB. He still maintains a connection with his former staff by virtue of his new position and with a continued interest in the laboratory work. Dr. Windle was succeeded by Dr. Palay, who serves both as chief of the Laboratory and as chief of the Section on Neurocytology. As a part of the reorganization accompanying this change in command, the Section of Perinatal Physiology in Puerto Rico has been shifted out of the Laboratory of Neuroanatomical Sciences to the office of the Assistant Director. Dr. Lloyd Guth has been appointed acting chief of the Section on Development and Regeneration, succeeding Dr. Windle in this position.

As in former years the investigations of this Laboratory are so various that they are best summarized under the specific Sections. Separate accounts are given below for each of the four active Sections.

Section on Neurocytology

S. L. Palay, Chief

As in previous years, the work of this section has been divided between chemical and morphological problems. In the latter category, we have carried out the first successful electron microscopic examination of the perikarya of neurosecretory neurons. This was accomplished in the preoptic nucleus of the goldfish where the neurosecretory substance appears as droplets of two sizes. The larger droplets, 1 or larger in diameter, appear to originate from multivesicular bodies and resemble, in their fine structures, the lysosomes of liver and kidney cells. The small droplets, 0.1 in diameter, appear to arise in the vesicles and cisternae of the Golgi complex. The distinctions between the two types of droplets suggest that they may have different functions.

Together with Dr. Jack Rosenbluth, we have studied the myelinated neurons of the eighth nerve ganglia in goldfish and the rat. These nerve cells are surrounded by a layer of myelin similar to the myelin sheath around large axons. In the fish the sheath varies in thickness and compactness, whereas in the rat it is usually thin and compact. The presence of the sheath poses certain questions regarding the nutrition and electrical activity of these neurons.

Work in progress includes electron microscopic studies of the ependyma lining the ventricles of the brain in fish and rats, and the fine structure of autonomic ganglia in the rat's intestine.

Dr. Albers has been studying the properties of succinic semialdehyde dehydrogenase purified from monkey brain. The affinity of the enzyme for the substrate is extremely high and probably accounts for the small concentration of succinic semialdehyde in the brain.

Dr. Spencer Gordon left the Section in July to resume clinical training at the University of Rochester.

The section was host this year to Dr. John Hills, NINDB trainee, and Dr. Mary Grillo and Dr. David Wolfe, PHS research fellows. Dr. L. Embree has served as Research Associate with Dr. Albers.

Dr. Albers presented a report of his work at the Fourth International Symposium on Neurochemistry in Varenna, Italy and Dr. Palay presented a report at the European Regional Conference on Electron Microscopy at Delft, The Netherlands, at which he was also chairman of the session of nerves and sensory organs. Dr. Palay also lectured at the Symposium on The Impact of Electron Microscopy on Biology at the AAAS annual meeting in Chicago; the Department of Biology, University of Maryland, College Park; the Army Graduate Medical School, Walter Reed Army Medical Center, and the Albert Einstein Medical College, New York. He was also chairman and organizer of the Fourth Gordon Conference on Cell Structure and Metabolism (Secretion) in Meriden, New Hampshire.

Section on Experimental Neuropathology

J. Cammermeyer, Chief

The main research objectives of the Section on Experimental Neuropathology have been to study the normal structure of the brain and spinal cord in various animals in order to establish a baseline for experimental studies.

Annual Reports (1960)

As part of continued interest in perfecting the techniques of tissue preservation, Dr. Mignon Malm tested in rats and guinea pigs a new fixative, P-toluenesulfonic acid, the usefulness of which is highly recommended because of its rapid action and the excellent histological results obtained; a preliminary report was presented before the 7th International Congress of Anatomists, New York, April 1960 (Anat. Rec., 136: 237, 1960).

When microscopic sections of the central nervous system were studied it became apparent that the absence of "dark" neurons and "pyknotic" nuclei could be used as an index of perfect fixation. In order to formulate principles for the perfect preparation of tissues, a number of experiments were performed to elucidate the factors involved; these artifactual changes were due to the lack of attachments between cellular membranes and their surroundings. Accordingly a procedure was developed whereby postmortem traumatization no longer severed the attachments; organs must be fixed by perfusion and autopsied after a delay of several hours. Only such material should be used for critical cytological studies, or for comparative anatomical and pathological studies.

A study of the cellular and vascular arrangements in well preserved tissues of primates, carnivores, rodents, and marsupials disclosed three new factors which may prove significant for a better understanding of normal physiological processes and may clarify the pathogenesis of focal damage. (1) All neurons throughout the central nervous system contact one or several blood vessels; this intimate neuronal-vascular contact may enable direct exchange of substances. (2) Oligodendrocytes are arranged perivascularly in a manner which varies with region and species; this unique distribution could reflect a function similar to intrinsic blood flow control to neurons, reported before the 35th Annual Congress of the Pan American Medical Association, Mexico City, May 1960. (3) Bundles of connective tissue fibers traverse the nervous tissue to form attachments between adjacent blood vessels; these intervascular fibers should be important for anchorage of the vessels.

Experiments in Macaca cynomolgus were planned to demonstrate anatomico-functional correlations. One of these series utilized psycho-pharmacological agents as Serpasil and Amphetamine. Apart from moderate changes in the content of basophil material within Purkinje cells no permanent damage had occurred. A brief review of results was presented at the Symposium on the Current Status of Parkinson's Disease, the Parkinson's Disease Foundation, Inc., in New York, March 1960.

Study of experimental dog material disclosed a high incidence of meningo-encephalitis with severe hydrocephalus, reported before the Annual Meeting of American Association of Neuropathologists, Boston, June 1960. As a consequence this species has been rejected for neuropathological studies. The frequent occurrence of meningo-encephalitis in all other animal species, too, should obligate the investigator to interpret all his results with care.

Jan Cammermeyer has since September 1960 been a member of the NINDB Editorial Committee.

The Section on Experimental Neuropathology was host to Dr. Mignon Malm until February 1960, when she accepted a research fellowship in Princeton.

Section on Development and Regeneration

Lloyd Guth, Acting Chief

The following changes in personnel occurred during the last year: Dr. William F. Windle, chief of the section, resigned to accept the post of Assistant director of NINDB; Dr. Lloyd Guth was appointed acting section chief. Dr. Clark J. Bailey resigned in order to take a position with the Division of Research Grants. The scientific staff, in addition to Dr. Guth, now consist of Dr. Earl R. Feringa and Dr. Jerald J. Bernstein. Dr. Feringa has been with the section for one year, and Dr. Bernstein has been here for three months.

The program of this section is the experimental analysis of the organization of the nervous system. In keeping with the philosophy of the previous section chief all scientists are accorded complete freedom in the choice and execution of their research program. However, all members of the section were selected because of a manifest interest in experimental neurology. Nerve regeneration provides a unique experimental tool for studying many different problems in neurology, e.g., cell biology, neuronal pathways of the central nervous system, and the functional organization of the nervous system. The aim of the section is not merely to study nerve regeneration, for that would constitute too restricted an approach to neurological investigation, but rather to focus on basic neurological problems, that may be approached experimentally.

Dr. Guth has been studying neuronal specificity in the central and peripheral nervous systems. Investigation of the anatomical and functional effects of heterogeneous nerve regeneration (i.e., regeneration of a nerve into a "foreign" termination) has proved a useful approach to the study of nerve specificities. His research had demonstrated that in the mammalian autonomic nervous system foreign nerve fibers can restore tonic function to organs such as the pupil or blood vessels, but there is no evidence that these foreign neurons are incorporated into pupillary or vascular reflexes. Consequently, it appears that the mammal is incapable of altering its intracentral pathways in response to alterations of nerve-end organ relationships. If this is true, then how can we account for functional recovery after direct suturing of transected peripheral nerves? Most peripheral nerves are composed of thousands of nerve fibers subserving many

different functions. If nerve regeneration is random (governed solely by mechanical factors) then the possibility that a given fiber will reach its appropriate termination is very remote indeed. Inasmuch as central nervous system reorganization is so limited in mammals, we must postulate some neurotropic mechanism by which nerve fibers achieve selective regeneration. Dr. Guth is presently studying this problem.

It is well known that regeneration of the central nervous system occurs only in certain infra-mammalian species. Largely as a result of Dr. Windle's research we have come to realize that the impotence of regenerative capacity in the mammalian central nervous system results partially from the mechanical blockade of the glial scar. However, we cannot rule out the possibility that mammalian neurons themselves suffer from an intrinsic inability to regenerate cut nerve processes. The inverse relationship between the phylogenetic position and the regenerative capacity of the central nervous system could be accounted for by phylogenetic differences in either the glia or the neurons. The role of the glia and of the neurons are currently being investigated by Dr. Earl Feringa.

Dr. Bernstein proposes to take advantage of the regenerative capacity of lower forms (e.g., fish) in order to study the physiology of such special senses as vision, gustation, and olfaction. The use of ablation techniques in mammals has been restricted by difficulties of interpretation. That the loss of a cerebral area results in loss of a specific function does not prove that this area governs that function. The brain is far too complex an organ to function in so simple a fashion. The fish is a more felicitous choice for ablation studies. In this animal one can both observe deficit function after ablation and study the changes that occur as the ablated part regenerates. Furthermore, function can be studied by allowing fibers to regenerate into foreign cerebral areas (i.e., by rearranging cerebral pathways). This "phylogenetic-regenerative" approach has great potential for extending our knowledge of the functional organization of the central nervous system in all species of animals.

Section on Functional Neuroanatomy

G. L. Rasmussen, Chief

During 1960 Dr. Gacek, a Research Associate, and Dr. Walther left the section. Dr. Gacek is receiving training in clinical otology under Dr. Schuknecht of Henry Ford Hospital; and Dr. Walther, a visiting scientist sponsored by the National Academy of Sciences, returned to the University of Goettingen, Germany, after having worked 16 months in this section. Dr. Morest, who had previously worked here during two three-month periods under COSTEP, rejoined the section as a member of the PHS Commissioned Corps.

Annual Reports (1960)

Research activities of the section have been confined almost entirely to study of (a) the manner of innervation of the receptor organs of hearing and balance (vestibular) and (b) the central conduction circuits of the auditory system.

The problem concerning the ultimate termination of efferent fibers of both the vestibular and cochlear nerves has been reinvestigated by means of the acetylcholinesterase histochemical method. Our previous studies in which various fiber degeneration methods were employed, could only demonstrate that the efferents of either nerve entered the sensory epithelium. The histochemical method has a unique advantage for the study of distribution of the efferent fibers since they possess a much higher degree of acetylcholinesterase activity than do the afferents. Due to improvement of the histochemical method of Koelle which offers a greater degree of localization of the enzyme to the axons and their endings, it has been possible to follow the selectively colored efferent fibers of the vestibular and cochlear nerves from the brain to the epithelium of all the receptor organs of the inner ear (chinchilla and cat). In the organ of Corti the efferents are easily traceable to the inner and outer hair cells; fibers to the outer hair cells have been observed to bend sharply apicalwards and course a considerable distance, winding in and out among the rows of receptor cells. Conceivably each fiber could therefore make synaptic contact with numerous hair cells, but this remains to be proven with a more efficacious method of approach such as electron microscope studies of the organ of Corti which has been previously deprived of its efferent innervation.

A study of this design is to be carried out with Dr. C. Smith of St. Louis, who has for several years been studying with the electron microscope the innervation of the organ of Corti in the normal guinea pig. Electron micrograph studies by others thus far show a disproportionately large number of efferent type endings in respect to relatively few afferent fibers. It is certain however, that these terminal fibers are of the efferent bundle originating in the brain, since all traces of acetylcholinesterase activity disappear from the organ of Corti 7 to 10 days after cutting the bundle in the medulla of the cat and chinchilla.

Additional information has been gained concerning the efferent innervation of the vestibular receptors by means of histochemical studies. Previously Rasmussen and Gacek demonstrated an efferent innervation of all vestibular receptors with the aid of fiber degeneration methods, and Prof. Dohlman, a visiting scientist of this section, demonstrated a high concentration of acetylcholinesterase in some fibers located next to hair cells of these receptor organs in the normal pigeon. Recently we have shown experimentally in the pigeon and chinchilla the disappearance of the histochemical reaction of these terminals in all the vestibular receptors following transection of the efferent bundle in the medulla--the bundle which distributes its fibers to all receptors of the inner ear.

The establishment of two types of fiber innervation (afferent and efferent) of the receptors of their differing chemical nature would have important physiological significance.

Dr. Dohlman, retired professor of Otolaryngology of Lund, Sweden, rejoined the section to continue his studies related to the physical and physiological conditions involved in stimulation of the hair cells of the semi-circular canals in particular, and the mechanism of hair cell stimulation of all receptors of the inner ear. Professor Dohlman is a very active worker and a world recognized authority on the vestibular apparatus. After leaving the section April 30, 1960 he presented a paper at the symposium held at the University of Basel, Switzerland; another, "Physiology of the Endolymph" at the Scandinavian Otolaryngological Congress, Stockholm; and a third paper "The Mechanism of Hair Cell Stimulation" before the Collegium Otolaryngologicum at Padua, Italy. Dr. Dohlman was responsible for the organization of the Symposium at Padua together with Prof. Arslan of Italy.

Robert Boord, formerly a graduate student of the University of Maryland, received his Doctorate degree in January 1960. The research work comprising both his M. S. and Ph. D. theses was carried out entirely in this section. He will be leaving the section in July after termination of his Postdoctoral Fellowship for a position elsewhere. Dr. Boord's study on the comparative anatomy of the efferent cochlear bundle of submammalian animals is particularly noteworthy.

Studies of the auditory circuitry between the levels of the inferior colliculus, medial geniculate and auditory cortex have been investigated during the past 18 months. Anatomical studies of the fiber connections of these areas have been neglected during the past 50 years.

Studies on the distribution of fibers arising from the inferior colliculus have disclosed two noteworthy findings. Contrary to previous evidence and prevailing ideas, not all of the ascending fibers from the colliculus course in the brachium to the medial geniculate body nor do all fibers entering the geniculate body terminate in all portions of it. Many fibers leave the brachium of the inferior colliculus to ascend in the reticular formation of both sides to undetermined destinations; other fibers course outside of the principal auditory pathway and terminate in the subthalamic nucleus. Most surprising was the finding that the auditory fibers of the brachium, the principal auditory pathway to the geniculate body, apparently terminate only in the inferior portion of the latter, a portion previously designated as the inferior lobe by Cajal. The dorsal portion or superior lobe (Cajal) remains strikingly free of fiber and synaptic degeneration following destruction of the inferior colliculus. This result has led us to investigate the nature of the superior lobe and in particular to determine whether other fibers of other sensory modalities may be carried into the auditory pathways at this level.

In this project most of the delicate brain operations requiring manual placement of small lesions in the auditory pathways were performed most skilfully by Dr. Gacek.

A study of the feedback connections from the five currently recognized auditory cortical areas to different cell groups of the medial geniculate body has been completed with the help of J. Walther. The significance of this corticofugal circuit is unknown, however, the anatomical information obtained should serve as a basis for future physiological studies and interpretation of results.

At the invitation of the Montreal Neurological Institute, Dr. Rasmussen delivered a lecture on "Normal morphological and experimental studies on the synapse," November 16, 1960.

Other Activities, Laboratory of Neuroanatomical Sciences

The scientists of the Laboratory of Neuroanatomical Sciences have been called upon to participate in activities not directly related to conducting experiments. Several serve on committees and advisory panels.

Dr. Brightman is President of the Washington Society for Electron Microscopy for the year 1960-61. Dr. Palay was Secretary of the NIMH-NINDB Assembly of Scientists and a member of the Cell Biology Study Section.

Dr. Rasmussen serves as a member representing NIH on the Committee of Hearing and Bio-acoustics, Armed Forces Research Council.

Dr. Lloyd Guth is a member of the Anatomy and Physiology Fellowship Panel, DRG. He lectured at the Department of Biology, Howard University, and at Creedmore Institute for Psychobiologic Research, Long Island.

Editorial tasks have engaged some of the investigators' time during the year. Dr. Rasmussen has completed editing of the monograph entitled "Neural Mechanisms of Auditory and Vestibular Systems" to be published shortly by Charles C Thomas. This is sixth in a series of "Symposia in Neuroanatomical Sciences" co-edited by W. F. Windle. Dr. Palay is on the editorial board of "Experimental Neurology." Dr. Cammermeyer has been a member of the NINDB Editorial Committee since September 1960.

Published Papers

1. Albers, R. W. The metabolism of γ-aminobutyrate. Chapter in: Amino acids and nucleotides in the central nervous system, R. O. Brady, and D. B. Tower, eds., Wiley, pp. 146-158, 1960.

2. Albers, R. W. The distribution of γ-aminobutyrate and related enzyme systems. Chapter in: Inhibition in the Nervous System and γ-aminobutyric Acid, E. Roberts and A. Van Harreveld, eds., Pergamon, pp. 196-201, 1960.

3. Albers, R. W., and W. B. Jakoby. Transaminations and the isotopic labelling of glutamate in brain. Ibid., pp. 468-470.

4. Albers, R. W., and W. B. Jakoby. Exchange reactions catalysed by γ-aminobutyric-glutamic transaminase. Biochem. Biophys. Acta, 40: 457-461, 1960.

5. Axelrod, J., R. W. Albers, and C. Clemente. The distribution of catechal-O-methyl transferase in the nervous system and other tissues. J. Neurochem., 5: 68-72, 1959.

6. Cammermeyer, Jan. A comparative study of intervascular connective tissue strands in the central nervous system. J. Comp. Neur., 114: 189-208, 1960.

7. Cammermeyer, Jan. Differences in shape and size of neuroglial nuclei in the spinal cord due to individual, regional and technical variations. Acta Anat. Basel, 40: 149-177, 1960.

8. Cammermeyer, Jan. The post-mortem origin and mechanism of neuronal hyperchromatosis and nuclear pyknosis. Exp. Neur., 2: 379-405, 1960.

9. Guth, L. Translation of Studies on Vertebrate Neurogenesis by S. Ramón y Cajal. Springfield, Ill., Charles C Thomas, 1960.

10. Guth, L., and C. J. Bailey. Effects of unilateral sympathectomy on the contralateral pupil of the cat. Exp. Neur., 2: 90-96, 1960.

11. Guth, L., L. Soutter, K. Frank, J. B. Campbell, and J. B. Lloyd. Diaphragmatic function following anastomosis of recurrent laryngeal and phrenic nerves. Exp. Neur., 2: 251-260, 1960.

12. McKhann, G. W., R. W. Albers, L. Sokoloff, O. Mickelsen, and D. B. Tower. The quantitative significance of the -aminobutyric acid pathway in cerebral oxidative metabolism. Chapter in: Inhibition in the Nervous System and γ-aminobutyric Acid, E. Roberts and A. Van Harreveld, eds., Pergamon, pp. 169-181, 1960.

13. Palay, S. L., and Leonard J. Karlin. On the appearance of absorbed fat droplets in the nuclear envelope. J. Biophys. Biochem. Cytol., 7: 391-392, 1960.

14. Portin, Bertram A., Grant L. Rasmussen, John D. Stewart, and Murray N. Andersen. Physiologic and anatomic studies thirty-five months after successful replantation of the lung. Jour. Thor. and Cardiovascular Surgery, 39: 380-384, 1960.

15. Ramsey, H. Fat in the epidural space in young and adult cats. Am. J. Anat., 104: 345-379, 1959.

16. Ramsey, H. Comparative morphology of fat in the epidural space. Am. J. Anat., 105: 219-232, 1959.

Papers in Press

1. Albers, R. W., G. Koval, G. McKhann, and D. Ricks. Quantitative studies of *in vivo* γ-aminobutyrate metabolism. J. Neurochem. Suppl. (in press).

2. Boord, Robert. The efferent cochlear bundle in the caiman and pigeon. Exp. Neur. (in press).

3. Cammermeyer, Jan. The frequency of chronic meningoencephalitis with hydrocephalus in dogs. J. Neuropath. Exp. Neur. (in press).

4. Cammermeyer, Jan. Reappraisal of the perivascular distribution of oligodendrocytes. Am. J. Anat. (in press).

5. Cammermeyer, Jan. Is the perivascular oligodendrocyte another element controlling the blood supply to neurons? Seventh Conference on Microcirculatory Physiology and Pathology. Trans. Microcirc. Conf. (in press).

6. Cammermeyer, Jan. An evaluation of the significance of the "dark" neurons. Ergeb. Anatomie (in press).

7. Gacek, Richard R. Efferent component of the vestibular nerve. Chapter 21 in "Neural Mechanisms of the Auditory and Vestibular Systems," G. L. Rasmussen and W. F. Windle, eds., Charles C Thomas (in press).

8. Gacek, R. The macula neglecta in the feline species. J. Comp. Neur. (in press).

9. Gacek, Richard R., and G. L. Rasmussen. Fiber analysis of the statoacoustic nerve in guinea pig, cat, and monkey. Anat. Rec. (in press).

10. Guth, L., and C. J. Bailey. Role of the sympathetic and parasympathetic systems in the consensual pupillary darkness reflex. Exp. Neur. (in press).

11. Guth, L., and C. J. Bailey. Pupillary function after alteration of the preganglionic sympathetic innervation. Exp. Neur. (in press).

12. Palay, S. L. Fine structure of neurosecretory cells in the preoptic nucleus of fishes. Proceedings of the European Regional Conference on Electron Microscopy, Delft, 1960. (In press)

13. Palay, S. L. The fine structure of secretory neurons in the preoptic nucleus of the goldfish (*Carassius auratus*). Anat. Rec. (in press).

Papers in press (cont'd)

14. Rasmussen, Grant L. A method of staining the statoacoustic nerve in bulk with Sudan black B. Anat. Rec. (in press).

15. Rasmussen, Grant L. Efferent fibers of the cochlear nerve and cochlear nucleus. Chapter 8 in "Neural Mechanisms of the Auditory and Vestibular Systems," G. L. Rasmussen and W. F. Windle, eds., Charles C Thomas (in press).

Preliminary papers, Abstracts and Discussions

1. Boord, R. L., and G. L. Rasmussen. Experimental demonstration of an efferent cochlear bundle in the pigeon. Anat. Rec. 136: 167, 1960.

2. Cammermeyer, Jan. Distribution of oligodendrocytes in cerebral gray and white matter of several animals. Am. J. Anat.

3. Guth, L. and C. J. Bailey. Some effects of unilateral sympathectomy on the contralateral and ipsilateral pupils. Anat. Rec., 136: 203, 1960.

4. Malm, M. Introduction of p-toluenesulfonic acid for perfusion-fixation of the central nervous system. Anat. Rec. 136: 237, 1960.

5. Rasmussen, G. L., R. Gacek, E. McCrane, and C. Baker. Model of the cochlear nucleus (cat) displaying its afferent and efferent connections (demonstration). Anat. Rec., 136: 344, 1960.

6. Rosenbluth, Jack, and S. L. Palay. Electron microscopic observations on the interface between neurons and capsular cells in dorsal root ganglia of the rat. Anat. Rec. (in press).

7. Rosenbluth, Jack, and S. L. Palay. The fine structure of myelinated bipolar neurons of the eighth nerve ganglia in the goldfish. Anat. Rec. (in press).

8. Walther, Jost B., and Grant L. Rasmussen. Descending connections of auditory cortex and thalamus of the cat. Fed. Proc. 19: 189, 1960.

Serial No. NINDB-NS-NC-1
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Ultrastructure of nervous system.

Principal Investigator: Sanford L. Palay

Other Investigators: Spencer Gordon, Jack Rosenbluth, Milton W. Brightman

Cooperating Units: None

Man Years (calendar year 1960):
Total: 8.0
Professional: 4.0
Other: 4.0

Project Description:

Objectives: To study the organization of nervous tissue at the level of fine structure, particularly the relations between nerve cells and between the several cell types of supporting cells and the nerve cells.

Methods employed: The principal instrument employed is the electron microscope. Tissues are prepared for examination by fixation in osmium tetroxide, embedding in methacrylate, araldite, or epon, and sectioning. The sections are mounted on carbon-coated grids, stained with lead salts, and examined in the electron microscope. Thicker sections are mounted in glycerine and examined in the phase contrast microscope.

Major findings: A study of neurosecretory cells in the preoptic nucleus of the goldfish was carried out as a preliminary to study of the corresponding neurons in mammals. This is the first successful examination of secreting neurons that has yet been reported. The nerve cells of the preoptic nucleus are disposed in clusters separated by ependymal processes. The neurons in each cluster are closely packed together. Individual cells display marginated Nissl substance, eccentric nucleus, and a central zone containing the Golgi complex, mitochondria, neurosecretory droplets, and multivesicular bodies.

Part A Project Description (cont'd)

The secretory material appears in the form of dense droplets of two sizes, 0.1 μ and 1 μ or larger. No intermediate stages between the two types of droplets were seen. Each droplet is enclosed in a single membrane, 60 Å thick, and has a fine granular texture. It is suggested that the small droplets arise primarily in the cisternae of the Golgi complex, whereas the large droplets originate by gradual transformation of the multivesicular bodies. The distinctions between the two types of droplets may indicate that they have different chemical compositions and different functions.

In collaboration with Dr. Brightman an electron microscopic study of the ependyma in fishes and rats has been initiated. Despite certain technical problems, the study has already revealed important structural differences between the cells in these two classes of vertebrates. Ependymal cells in the rat are nearly filled with fine filaments oriented in a roughly spherical pattern around the nucleus. In the goldfish these filaments are infrequent and instead the cytoplasm is occupied by large numbers of tubules and vesicles. An additional difference involves the ciliary apparatus. In the rat the basal corpuscles of the cilia are fitted with a bundle of short thin filaments that extend at a slight angle from the corpuscle. In the goldfish each basal corpuscle is fitted with a pair of long, cross-striated bands consisting of fine filaments and extending from the basal corpuscle in a plane perpendicular to the longitudinal axis of the cilium, apparently to insert into the terminal bars at the lateral cell border.

In collaboration with Dr. Rosenbluth, the study of myelinated neurons in the eighth nerve ganglia of the goldfish has been completed and has been extended to the corresponding cells in the spiral and vestibular ganglia in the rat. In this mammal, the myelinated neurons have rather thin sheaths consisting of only 5 - 10 myelin lamellae, whereas in the goldfish the sheaths can be 50 or more lamellae thick. Dr. Rosenbluth has also investigated the effects of stimulation and of certain drugs upon the surface membrane of dorsal root ganglia in the toad. The results of this study are, however, too preliminary for summary at this time.

Together with Dr. Grillo, who is a guest in this Section, a study of the autonomic ganglia in the stomach and intestine has been begun and is still in an exploratory stage. Dr. Wolfe is initiating a study of the fine structure in developing dorsal root ganglia in the chick.

Significance: These studies deal with the ultrastructural basis of neural action. The project now includes investigation of neural tissues from the central, peripheral, and autonomic nervous systems. The neuron as a transmitting unit and as a secreting unit is being considered. The relationship between neurons and between so-called supporting structures

Part A Project Description (cont'd)

and neurons is being pursued by studies of ependyma, glia, and Schwann cell sheaths. These investigations will provide a structural basis for the complex physiological interrelations among all of these elements.

Proposed course of project: The work will be continued by further investigations of glia in the central nervous system, of autonomic ganglia in the intestine, and of the ependymal lining of the third ventricle. Additional studies of the dorsal root ganglion in different physiological states are necessary to complete the work already done.

Part B included: Yes

Serial No. NINDB-NS-NC-1

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Palay, S. L.: Fine structure of neurosecretory cells in the preoptic nucleus of fishes. Proceedings of the European Regional Conference on Electron Microscopy, Delft, 1960. To be published January 1961.

Palay, S. L.: The fine structure of secretory neurons in the preoptic nucleus of the goldfish (Carassius auratus). Anat. Rec. December 1960 (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-NS-NC-2
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project title: The role of γ-aminobutyric acid in the metabolism of the central nervous system.

Principal Investigator: R. W. Albers

Other Investigators: L. Embree, G. Koval

Cooperating Units: None

Man years (calendar year 1960):
Total: 3.0
Professional: 3.0
Other: 0

Project Description:

Objectives: To assess the functional role of γ-aminobutyric acid in the central nervous system.

Methods employed: Isotope studies, classical enzyme fractionation techniques, cytological fractionation, spectrophotometric and fluorimetric measurements.

Major findings: The in vivo isotope studies, previously reported, have been concluded and the results were reported at the Fourth International Symposium. The extremely high apparent metabolic turnover of γ-aminobutyrate is ascribable to heterogenous metabolic pools of brain glutamate.

The investigation of transaminase activities was extended in collaboration with Dr. W. Jakoby of NIAMD. Part of this work has been published and a further manuscript is in preparation.

Succinic semialdehyde dehydrogenase was first described by us in 1959. The enzyme has now been purified from monkey brain and its properties are being studied intensively. This work has considerably advanced and part of it is being prepared for publication. The initial observations have been confirmed; the affinity of the enzyme for the substrate considerably exceeds the original estimate. This accounts for the extremely small

Part A Project Description (cont'd)

concentration of succinic semialdehyde endogenously present in brain. Several properties of the enzyme are of interest in relation to the general mechanism of action of aldehyde dehydrogenases and also for the possible physiological interaction with certain other brain metabolites which have neurohumoral associations. Elucidation of these aspects of the enzyme is, at present, the major effort of the reporting investigator.

Significance: This was developed at length in the 1959 report.

Proposed course of project: In addition to continued pursuit of aspects of the project in progress, related studies of the phylogenetic distribution and cytochemical localization of the enzyme are under consideration for the future.

Part B included: Yes

Serial No. NINDB-NS-NC-2

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Albers, R. W., and W. B. Jakoby. Exchange reactions catalysed by γ-aminobutyric-glutamic transaminase. Biochem. Biophys. Acta, 40: 457-461, 1960.

Albers, R. W., G. Koval, G. McKhann, and D. Ricks. Quantitative studies of in vivo γ-aminobutyrate metabolism. J. Neurochem. (in press).

Albers, R. W. The distribution of γ-aminobutyrate and related enzyme systems. Chapter in: Inhibition in the Nervous System and γ-aminobutyric Acid, E. Roberts and A. Van Harreveld, eds., Pergamon, pp. 196-201, 1960.

Albers, R. W., and W. B. Jakoby. Transaminations and the isotopic labelling of glutamate in brain. Ibid., pp. 468-470.

McKhann, G. W., R. W. Albers, L. Sokoloff, O. Mickelsen, and D. B. Tower. The quantitative significance of the γ-aminobutyric acid pathway in cerebral oxidative metabolism. Ibid., pp. 169-181.

Axelrod, J., R. W. Albers, and C. Clemente. The distribution of catechal-O-methyl-transferase in the nervous system and other tissues. J. Neurochem., 5: 68-72, 1959.

Serial No. NINDB-NS-EN-3
1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The pathogenesis of myelopathies.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 2.4
Professional: .4
Other: 2.0

Project Description:

Objectives: To determine factors responsible for localization and progression of lesions in the spinal cord of animals with application to human myelopathies.

Methods employed: Measure volume of spinal cord in different animal species preferably of known age, fix the organ by perfusion, delay the autopsy, prepare microscopic sections routinely and stain them by the periodic-acid Schiff treatment combined with gallocyanin-chrome alum.

Major findings: (1) Volumetric and histologic studies of material fixed by different procedures revealed that uniform and excellent histologic results were obtained after use of a rapid coagulant fixative as Heidenhain's Susa solution, provided the autopsy was delayed 4 hours. A similar useful application of p-toluene sulfonic acid was tested and described by Dr. Mignon Malm (Anat. Rec., 136: 237, 1960).

(2) Artifactual neuronal changes as "dark" neurons, the presence of which obscures normal and pathologic cells, can be avoided by the technique developed here. To understand the mechanism involved, a series of experiments was performed to either avoid or produce "dark" neurons; the hypothesis was formulated that these cells are formed by the post-mortem severance of delicate attachments between membranes and their surroundings. The action of a "good" fixative is to affix the membranes permanently.

Unwanted changes of myelinated sheaths can not be prevented.

Part A Project Description

An unusual cell type occurred which stained diffusely pink with the periodic-acid Schiff technique; the significance of this cell type remains unknown.

(3) All animal material was studied microscopically to determine the degree of spontaneous diseases among animal species; dogs suffered from meningo-encephalitis in 100 per cent, complicated with hydrocephalus in 50 per cent. Accordingly this species is not utilized for experimental studies of the brain and spinal cord.

(4) The external structures of the spinal cord were studied in various animals. The extradural fat, according to Dr. Helen D. Ramsey, is distributed in a complex manner in both young and old animals; these observations appeared in 1960 (American Journal of Anatomy, vol. 104: 345-380; vol. 105: 219-232, 1959).

(5) The arrangement of cells and vessels of the CNS from various mammalian species was found to be alike in all species except the marsupial; the marsupial is known to have a peculiar vascular system of capillary loops and is now found to be free of intervascular strands of connective tissue fibers. The presence of these strands in all other species should not be confused with reactive vascular changes. They may be important for normal "anchorage" of blood vessels. They may possibly localize disease processes when stretched and the vascular lumen is constricted.

Also noteworthy were observations on the close association between blood vessels and neurons as well as between blood vessels and oligodendrocytes. These results may prove significant for clarifying contemporary concepts about control mechanisms for exchange of substances, as well as factors involved in damage of brain and spinal cord.

Proposed course of project: Serial sections of samples taken from known levels of the spinal cord of experimental animals, using standard histological procedures developed here and recommended because the tissues hereby have a uniform appearance.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cammermeyer, J.: A comparative study of intervascular connective tissue strands in the central nervous system. J. Comp. Neur., 114: 189-208 (1960).

Cammermeyer, J.: The post-mortem origin and mechanism of neuronal hyperchromatosis and nuclear pyknosis. Exp. Neur., 2: 379-405 (1960).

Cammermeyer, J.: An evaluation of the significance of "dark" neurons. Ergebn. Anat. Entwicklungsgesch., 00: 000-000 (1960).

Cammermeyer, J.: Frequency of meningoencephalitis and hydrocephalus in dogs. J. Neuropath. Exp. Neurol., 00: 000-000 (1960).

Serial No. NINDB-NS-EN-4
1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The functional significance of oligodendrocytes.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 2.4
Professional: .4
Other: 2.0

Project Description:

Objectives: To determine the role of oligodendrocytes in normal and diseased organs.

Methods employed: To study the distribution of oligodendrocytes in different normal and experimental animal species by routine and special histological techniques.

Major findings: (1) The size and appearance of oligodendrocytes, as well as other cells of the central nervous system depend on the fixation procedure. Karyometric and histological results are optimal when the animals are perfused with a modified Heidenhain's Susa solution, preceded by removal of blood and then delaying the autopsy 4 hours.

(2) A comparative karyometric and microscopical study of several species revealed that the size of oligodendrocytes varies with both region and species.

(3) The oligodendrocytes tend to have an exclusive perivascular arrangement near neurons and perivascular arborizations of both grey and white matter.

(4) Differences in the distribution and number of oligodendrocytes occur, and for a comparison between normal and experimental or pathologic material, only sections from identical regions and species should be used.

Part A Project Description

(5) The conclusion of these studies refutes older concepts about the functional significance of oligodendrocytes. A new hypothesis is formulated that these cells form juxtavascular clusters which are concerned with intrinsic control of blood flow to neurons.

Proposed course of project: Studies of the perivascular organization of oligodendrocytes with various histological and histochemical techniques in normal and experimental animals.

Part B included: Yes

Serial No. NINDB-NS-EN-4

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cammermeyer, J.: Differences in shape and size of neuroglial nuclei in the spinal cord due to individual, regional and technical variations. Acta Anat., 40: 149-177 (1960).

Cammermeyer, J.: Is the perivascular oligodendrocyte another element controlling the blood supply to neurons. Angiology, 14: 000-000 (1960).

Cammermeyer, J.: Reappraisal of the perivascular distribution of oligodendrocytes. Am. J. Anat., 106: 000-000 (1960).

Cammermeyer, J.: The distribution of oligodendrocytes in cerebral grey and white matter of several animals. (in manuscript).

Serial No. NINDB-NS-EN-5

PHS-NIH
Individual Project Report
Calendar Year 1960

1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

Part A

Project Title: "Light" microscope studies of normal and diseased neurons.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.2
Professional: .2
Other: 1.0

Project Description:

Objectives: To identify the normal, fatigued, and acutely damaged neurons of perfectly fixed material from animals perfused with a coagulant fixative, and autopsied after a delay of several hours.

Methods employed: Histologic procedures developed in the Section on Experimental Neuropathology for preservation of tissue, are used.

Major findings: (1) Neurons in all regions contact blood vessels for varying lengths; their dependence on the blood flow control of oligodendrocytes is discussed in another project.

(2) Both normal and acutely damaged neurons are of large size, larger than conventionally described, also temporal changes of basophil material and cellular elements are at variance.

Proposed course of project: Animals kept under standard laboratory conditions (temperature, light, food) are exposed to different experimental conditions. Brains and spinal cords of monkeys, cats, rabbits and mice have been prepared histologically and readied for microscopical and karyometric examinations.

Part B included: No

Serial No. NINDB-NS-DR-6
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The specificity of the sympathetic nervous system in the regulation of pupillary function.

Principal Investigator: Lloyd Guth, M. D.

Other Investigators: C. J. Bailey

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.75
Professional: .25
Other: 1.50

Project Description:

Objectives: To determine whether non-pupillary sympathetic nerves can mediate pupillary sympathetic reflexes.

Methods employed: Transection of sympathetic rami T1 - T3 (pupillary rami) to allow collateral sprouts from T4 - T7 (non-pupillary rami) to innervate the pupillary post-ganglionic cells of the superior cervical sympathetic ganglion.

Major findings: Pupillary tonus is restored by the foreign nerve fibers, but these fibers do not participate in sympathetic pupillary darkness reflexes.

Significance: These results constitute further evidence that the mammalian central nervous system is relatively incapable of pathway reorganization after nerve transposition.

Proposed course of project: Completed.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Guth, L., and C. J. Bailey Effects of unilateral sympathectomy on the contralateral pupil of the cat, Experimental Neurology, 2: 90-96, 1960.

Guth, L. and C. J. Bailey Role of the sympathetic and parasympathetic systems in the consensual pupillary darkness reflex, Experimental Neurology, 1960, in press.

Guth, L., and C. J. Bailey Pupillary function after alteration of the preganglionic sympathetic innervation, Manuscript to be submitted to Experimental Neurology.

Serial No. NINDB-NS-DR-7
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Heterogeneous reinnervation of the diaphragm

Principal Investigator: Lloyd Guth, M. D.

Other Investigators: Karl Frank, Lamar Soutter, James B. Campbell, and John B. Lloyd.

Cooperating Units: Laboratory of Neurophysiology, NINDB, Boston University School of Medicine, and Columbia University College of Physicians and Surgeons.

Man Years: (calendar year 1960)
Total: 1.75
Professional: .25
Other: 1.50

Project Description:

Objectives: To determine whether diaphragmatic function can be maintained by the recurrent laryngeal nerve in rats and monkeys.

Methods employed: Anastomosis of proximal recurrent laryngeal and distal phrenic nerve segments.

Major findings: The recurrent laryngeal nerve can restore function to the denervated hemidiaphragm of the rat. In the monkey, the recurrent laryngeal nerve transmits functionally effective inspiratory action potentials to the denervated hemidiaphragm only during asphyxiation.

Significance: Some human diseases (e.g., "bulbar" poliomyelitis) are characterized by destruction of phrenic motoneurones. Reinnervation of the diaphragm by the recurrent laryngeal nerve may enable such patients to survive without the assistance of an artificial respirator. However, further clinical investigation is needed before this operation can be tried in man. The operation has the following potential

Part A Project Description (cont'd)

hazards: prolonged asphyxiation might produce diaphragmatic inspiratory spasm, tracheal irritation might elicit an inspiratory gasp instead of the protective cough reflex, and the degree of hypoxia necessary for the recurrent laryngeal nerve to be effective might be incompatible with life.

Proposed course of project: The experiment has been discontinued. The applied clinical aspects are being studied in Boston.

Part B included: Yes

Serial No. NINDB-NS DR-7

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications: Guth, Lloyd, L. Soutter, K. Frank, J. B. Campbell, and J. B. Lloyd. Diaphragmatic function following anastomosis of recurrent laryngeal and phrenic nerves, Experimental Neurology, 2:251-260, 1960.

Serial No. NINDB-NS-DR-8
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Regeneration of peripheral nerves.

Principal Investigator: Earl R. Feringa, M. D.

Other Investigators: J. B. Campbell, A. Bassett, C. Thuline

Cooperating Units: Department of Neurosurgery, Columbia University, College of Physicians and Surgeons.

Man Years (calendar year 1960)
Total: .25
Professional: .25
Other: 0

Project Description:

Objectives: To evaluate a nerve grafting procedure as a method of obtaining regeneration across relatively long gaps in peripheral nerves.

Methods employed: A segment of the sciatic nerve about two centimeters long was removed from goats and dogs. This gap in the nerve was filled with multiple segments of small, frozen, dried, irradiated nerves and the entire area wrapped with a Millipore cuff. Functional progress was recorded with motion pictures. Conduction across the area of the gap was determined electrophysiologically. Histological evaluation will be done.

Major findings: Functional return was noted in three of four dogs and in two goats. Transmission of nerve impulses across the graft was found in three dogs and one goat.

Significance: The preliminary results indicate that the described technique facilitates regeneration over long gaps.

Part A Project Description (cont'd)

Proposed course of project: This project has been discontinued at the National Institutes of Health and will be completed by Dr. Campbell at Columbia University.

Part B included: No

Serial No. NINDB-NS-DR-9
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Regeneration in the peripheral nervous system: Selectivity of pathways and specificity of terminations.

Principal Investigator: Lloyd Guth, M. D.

Other Investigators: Jerald J. Bernstein, Ph.D.

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.75
Professional: .25
Other: 1.50

Project Description:

Objectives: To determine whether peripheral nerves regenerate selectively to appropriate endings or randomly toward any termination.

Methods employed: 1. The sciatic nerve of the rat will be transected and resutured. After regeneration is complete the isometric tensions developed in the soleus and plantaris muscles upon stimulation of spinal roots L4 and L5 will be studied. A difference in the proportions of L4 and L5 fibers innervating these muscles will be evidence that regeneration was not random. 2. The cervical sympathetic trunk of the cat will be transected and resutured. After regeneration is complete, rami T1 - T7 will be stimulated. If T1 - T3 dilate the pupil and T4 - T7 constrict the ear vessels (as in the normal cat) then this will constitute evidence that regeneration has not been random. 3. In rats, spinal root L4 will be crushed so as to obtain collateral sprouting of L5 fibers in the soleus muscle. If regeneration of L4 results in the withdrawal of collateral sprouts of L5 (as determined from isometric tension measurements after stimulation of L4 and L5) this will indicate a specificity between nerve and muscle fibers. 4. In cats, sympathetic rami T1 - T3 will be crushed

Part A Project Description (cont'd)

to allow fibers T4 - T7 to sprout collaterals within the superior cervical sympathetic ganglion. If regeneration of T1 - T3 results in the withdrawal of the collateral sprouts (as determined by the effects of stimulating these rami) this will indicate that there is a specificity between the pupillary postganglionic cells and the pupillary preganglionic rami T1 - T3.

Major findings: None as yet.

Significance: Although the chemotropic hypothesis of Cajal has been discredited recently, no experiments have as yet resolved this issue. The proposed experiments may determine whether chemotropism plays a role in nerve regeneration

Proposed course of project: The project is being actively pursued and will constitute the major portion of the coming year's work.

Part B included: No

Serial No. NINDB-NS-DR-10
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Aspects of Experimental Neuroembryology

Principal Investigator: Lloyd Guth, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.75
Professional: .25
Other: 1.50

Project Description:

Objectives: To determine whether myotypic specification of neurons occurs in the adult newt.

Methods: Cross-anastomosis of spinal nerves III and IV (which are predominantly flexor and extensor respectively).

Major findings: None as yet.

Significance: Weiss' demonstration of the homologous response after limb transplantation in the larval urodele has raised many questions concerning the plasticity and organization of the nervous system. By continuing these studies ontogenetically and phylogenetically we hope to be able to partially resolve some of these problems.

Proposed course of project: Temporarily discontinued.

Part B included: No

Serial No. NINDB-NS-DR-11
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Regeneration in the spinal cord of cats and monkeys

Principal Investigator: Earl R. Feringa

Other Investigators: J. B. Campbell, A. Bassett, and C. A. Thuline

Cooperating Units: Department of Neurosurgery, Columbia University, College of Physicians and Surgeons.

Man Years (calendar year 1960)
Total: .25
Professional: .25
Other: 0

Project Description:

Objectives: To determine whether inhibition of vertebral mobility is beneficial to regeneration of the spinal cord.

Methods employed: Spinal fusions were done on cats. After obtaining roentgenographic evidence of successful fusion the spinal cord was transected. Spinal fusions were also done on thirteen monkeys. The animals have been sent to Columbia University for completion of the experiment.

Major findings: Spinal fusion was successful in both cats and monkeys. The effect of this procedure on cord regeneration has not as yet been evaluated.

Significance: If these studies demonstrate increased regeneration after spinal fusion we will have obtained evidence that movements of the transected cord after transection interfere with spinal cord axonal regeneration.

Proposed course of project: This project has been

Part A. Project Description (cont'd)

discontinued at NIH and will be continued by Dr. Campbell's group at Columbia University.

Part B included: No

Serial No. NINDB-NS-DR-12
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies to determine the possible role of auto-immune antigens in cerebral scar formation.

Principal Investigator: Earl R. Feringa, M. D.

Other Investigators: R. Wayne Albers, Ph.D.

Cooperating Units: None

Man Years (calendar year 1960)
Total: .25
Professional: .25
Other: 0

Project Description:

Objectives: It has been shown that mammals are allergic to intra-peritoneal injections of their own brain substance (allergic encephalitis studies). Apparently, the antigen contained in the brain is normally kept from the general circulation by the blood brain barrier. The proposed experiments investigate the possibility that the glial scar which blocks axonal regeneration following central nervous system injury may result from an auto-immune response to the release of brain antigen following the injury-provoked breakdown of the blood-brain barrier.

Methods employed: (a) Injection of brain substance during the neonatal period can make animals tolerant to homologous brain substance. Therefore, newborn rats were injected on the day of birth, and at regular intervals, thereafter, with small amounts of fresh, homogenized rat brain. After the animal had reached adult size the facial nerve was transected and the proximal end inserted into the cerebral cortex. Facial nerve regeneration and amount of glial scar formation will be compared in the experimental (brain injected) and control (untreated) groups.

Part A Project Description (cont'd)

(b) Inasmuch as 6-mercaptopurine and amethopterin have been shown to successfully inhibit auto-immune reactions, additional studies were undertaken in which adult animals received the same surgical procedure outlined above. The animals were treated post-operatively with 6-mercaptopurine or amethopterin. These animals will be similarly evaluated.

Major findings: None as yet.

Significance: If these experiments demonstrate that the major cause for formation of glial scar is an auto-immune reaction, then further efforts should be aimed at the bio-chemical prevention of the antigen antibody response and not at mechanical methods of minimizing the formation of glial scar.

Proposed course of project: Histological materials will be evaluated. Additional studies with inbred animals may be indicated.

Part B included: No

Serial No. NINDB-NS-DR-13
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of spinal cord regeneration in the newt

Principal Investigator: Earl R. Feringa, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: .25
Professional: .25
Other: 0

Project Description:

Objectives: To determine whether spinal cord regeneration in the newt results from regeneration of axons of preexisting neurons or from the axonal outgrowth of neuroblasts that have newly differentiated from ependymal cells.

Methods employed: At various times after transecting the spinal cord in newts the animals were injected with tritiated thymidine in order to label any dividing ependymal cells. The presence or absence of labeled neurons will be determined autoradiographically several months after transection.

Major findings: Injection of tritiated thymidine in newts approximately twelve days after cord transection results in the labelling of ependymal cells.

Significance: Tritiated thymidine is taken up by dividing cells only, and is retained indefinitely by the daughter cells. Consequently, the presence of labeled neurons in the fully regenerated newt spinal cord would constitute evidence that part, or all, of the regeneration of the newt spinal cord is the result of the formation of new neurons in response to spinal cord transection.

Part A Project Description (cont'd)

Proposed course of project: If this study indicates that the formation of new neurons plays a significant role in spinal cord regeneration in the newt, then the project will be extended to evaluate the possibility that the formation of new neurons is significant in the regeneration of the cord in other adult and larval animals.

Part B included: No

Serial No. NINDB-NS-DR-14
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The neurological basis of color vision in the fish

Principal Investigator: Jerald J. Bernstein, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: .50
Professional: .50
Other: 0

Project Description:

Objectives: To determine the neural pathways of color vision in the fish.

Methods employed: Electrolytic lesions will be made in specific areas of the fish brain. The operated fish will then be trained to make a hue discrimination using a conditioned autonomic response (cardiac rate) as the criterion for learning. Under this set of experimental conditions the conditioned autonomic response is a deceleration of heart rate upon presentation of a stimulus that has been formerly paired with shock. It has been found that normal fish will react to the wave-length characteristics of colored stimuli whereas forebrain ablated fish do not. If the electrolytic lesions cause a loss in the ability of the operated fish to make a hue discrimination it will then be known that the color visual pathway has been disrupted.

Significance: This study can establish the neural pathways of color vision in a primitive animal. The fish is being used because the neuroanatomical structure of the brain allows for manipulation of certain key visual centers without blinding the animal. The location of the color visual pathway can lead to a better understanding of this complex modality in all animals.

Part B included: No

Serial No. NINDB-NS-DR-15
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Gustatory function following heterogeneous reinnervation of taste buds in the fish

Principal Investigator: Jerald J. Bernstein, Ph.D.

Other Investigators: Lloyd Guth, M. D.

Cooperating Units: None

Man Years (calendar year 1960)
Total:
Professional:
Other:

Project Description:

Objectives: To determine whether functional central nervous system reorganization can occur after an area is innervated by a foreign cranial nerve.

Methods employed: Goldfish will be trained to make a gustatory discrimination (e.g., to quinine sulfate). By removal of the olfactory and gustatory cranial nerves it will be determined that the fish make this discrimination on the basis of taste and not smell. Various cranial nerve anastomoses will be performed (i.e., V to VII) on trained animals. These operated fish will be tested for their ability to make the gustatory discrimination. Parallel experiments will be run on olfaction. Correlative histological studies of taste buds and central gustatory centers will be performed.

Significance: This study can establish the ability of a region of the central nervous system to interpret sensory information from foreign sense organs. This group of experiments can lead to a greater insight into the problem of central nervous system organization.

Part B included: No

Serial No. NINDB-NS-FN-16
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Further studies on the efferent component of the vestibular nerve.

Principal Investigator: Richard R. Gacek

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: (1) To determine the manner in which the efferent vestibular fibers terminate, i.e., whether or not they end on the vestibular hair cells as the afferent vestibular supply does. (2) To locate the exact cells of origin of the efferent vestibular fibers.

Methods employed: The Sudan Black technique previously described by Rasmussen (1953) for study of Wallerian degeneration of nerves of the petrous bones is employed subsequent to production of lesions in the vestibular nuclei and other regions of the hindbrain. Serial sections of the vestibular root and all its peripheral branches including the receptor epithelium are studied in a large series of experimental animals (cats and chinchillas). Protargol silver, Glees' ammoniacal silver, and Nauta-Gygax axonal degeneration methods are employed to demonstrate the ultimate termination of the efferents.

The method of Brodal, which is based on the phenomenon of retrograde cell changes, is used to demonstrate the cell bodies of the efferent fibers.

Major findings: No major findings concerning the termination or exact cells of origin of the efferent vestibular fibers can be reported at this time. Original observations

Part A Project Description (cont'd)

dealing with the origin of the vestibular efferent fibers and their course and distribution in the labyrinth have been prepared for publication and will appear as a chapter in a monograph, "Neural Mechanisms of the Auditory and Vestibular Systems".

Significance: Experimental neuroanatomical description of the termination of the efferent vestibular systems is needed to verify the fact that endings possessing characteristics of centrifugal fibers have been described in the vestibular neuroepithelium by electron microscopic and histochemical means. Elucidation of the ultimate termination of these efferents will be of particular use in physiological study of this efferent system which may regulate sensory input, as the cochlear efferent bundle does in the cochlea.

Proposed course of project: Project dropped due to fact that principal investigator, Dr. R. Gacek left this section in May 1960 to take a position in the Department of Otolaryngology of Henry Ford Hospital. However, this problem is being pursued further by G. L. Rasmussen as part of another project.

Part B included: No

Serial No NINDB-NS-FN-17
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Comparative anatomy of the afferent and efferent auditory nerve connections in submammalian vertebrates.

Principal Investigators: Robert L. Boord

Other Investigators: Grant L. Rasmussen

Cooperating Units: This project is being conducted under the auspices of Public Health Service Post-doctoral Research Fellowship BF-9003 (C3), beginning May 1, 1960 under the sponsorship of Dr. G. L. Rasmussen, this laboratory.

Man Years (calendar year 1960):
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To study the efferent and afferent connections with regard to (1) the termination of efferent cochlear fibers and (2) the projection of afferent cochlear fibers to primary auditory centers in the central nervous system.

Methods employed: Small diverse lesions will be placed in the cochlear ganglion and fiber tracts disrupted in the brain stem as well as the peripheral system. Following necessary post-operative survival times, experimental axonal degeneration methods (Nauta-Gygax, Sudan black B, and protargol silver) will be utilized to reveal fragmented fibers emanating from lesion sites. Histochemical techniques for the localization of acetylcholinesterase will be employed in the study of efferent pathways and connections in both normal and experimental material.

Major findings: The initial phase of this study concerning the establishment of an efferent component of the reptilian and avian cochlear nerve has been completed. In both species the efferent bundle is similar in topographic

Part A Project Description (cont'd)

relationships to the efferent bundle previously described in mammals and therefore it is a homologous nervous structure which is probably present in all animals possessing a cochlea. More recent experiments indicate that the efferents terminate in the organ of Corti of the alligator and the pigeon.

Significance: The relationship between perception of auditory stimuli and the degree of development of the auditory system is not well understood. Knowledge of the less elaborate neuronal circuitry in the lower animal forms is needed in order to provide a basis upon which function and anatomical ideas of the mechanism of hearing can be advanced.

Proposed course of project: Study is to be continued until July 1, 1961 at which time the principal investigator's Postdoctoral Fellowship terminates.

Part B included: No

Serial No. NINDB-NS-FN-18
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The ascending and descending connections between the medial geniculate body and the auditory cortex.

Principal Investigator: Grant L. Rasmussen

Other Investigator: Jost B. Walther

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.25
Professional: 1.25
Other: 0

Project description:

Objectives: The object of this study is to (1) localize the areas of the cortex of cat that receive direct connections from the medial geniculate body and (2) establish which of the five cortical auditory fields connect corticofugally with the medial geniculate body.

Methods: Experimental neuroanatomical methods are employed. Following placement of small lesions in various locations of the medial geniculate body and the auditory cortical areas AI, AII, EP, insular and the temporal gyri, the resultant fiber and synapse degeneration is studied in Marchi, Nauta and the Rasmussen synapse preparations.

Major findings: Part (A) of project dealing with the geniculotemporal projection to the auditory cortex is not sufficiently complete to offer major findings at this time.

Part (B), concerning the cortical feedback connections of the geniculate body, has been completed and the essential finding appears in abstract form (Fed. Proc., v.19, Part I, p.189, 1960). The principal part of the medial geniculate body, except for its dorsal portion, received descending connections from auditory areas AI, AII, the posterior ectosylvian gyrus

Part A Project Description (cont'd)

(EP) and also from the more recently established auditory areas located in the insular and temporal cortex. The magnocellular part of the medial geniculate body, usually considered as a nonauditory cell group, also receives connections from the classical auditory cortex areas AI, AII and EP. In addition, these cortical areas project to other auditory nuclei of lower levels of the brain stem--the nucleus of the inferior colliculus of both sides and the dorsal nucleus of the lateral lemniscus of the same side.

Significance: Electrophysiological studies in recent years (Galambos, Desmedt and others) indicate that the descending system plays an important regulatory role over the incoming information traveling via the classical ascending conducting auditory pathways. The anatomical information disclosed about the corticogeniculate connections should serve as a basis for future physiological studies and interpretation of results that will lead to a better understanding of the functional role played by the feedback mechanism of the higher auditory level.

Proposed course of project: Part (A) concerning the geniculotemporal projection is to be completed during 1961. Part (B) concerning the corticogeniculate projection has been completed. Dr. Jost Walther - a visiting scientist from Germany played a major role in this investigation. He returned to Germany in April of this year.

Part B included: No

Serial No. NINDB-NS-FN-19
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: A study of the auditory and vestibular afferent and efferent systems including the receptors.

Principal Investigator: Grant L. Rasmussen

Other Investigators: Robert L. Boord
Gosta Dohlman

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To continue to explore and reveal unknown anatomical neuronal connections of the afferent and efferent divisions of the auditory system; to learn something about the anatomical and functional interrelationships of these two systems. The immediate problems under study are: (1) Establishment of the ultimate termination of the efferent fibers of both the cochlear and vestibular nerves which up to the present have been traced only as far as the respective receptor organs. (2) To study the morphology and function of the tectorial membrane and the cupulae of the hearing and vestibular apparatuses in order to understand the mechanism of hair cell stimulation. (3) To establish the ascending auditory connections of the inferior colliculus.

Methods employed: Since both the efferents of the vestibular and cochlear nerve exhibit a much higher concentration of acetylcholinesterase than the afferent fibers the histochemical method of Koelle (which we have modified to advantage) is employed to demonstrate the distribution and terminal relationship of the efferent fibers to the receptor cells of the receptor organs. (2) The use of tritium labeled glycin is employed in the study of the tectorial membrane and

Part A Project Description (cont'd)

cupula. Development of these structures in chick embryo and the process of reconstruction of the cupula in the adult after having previously removed it is also studied. (3) The newer and more effective techniques are chiefly depended upon for demonstration of axonal, preterminal (Nauta-Gygax method) and terminal or synaptic degeneration (Rasmussen method), as well as Golgi preparations. The employment of the experimental techniques on subjects in which small isolated lesions are properly placed makes it possible to reveal important complexly arranged neuronal connections.

Major findings: (1) Thanks to an improvement of the histochemical method of Koelle which offers a greater degree of localization of the acetylcholinesterase to the nerve axons and their endings it has been possible to follow the efferent fibers of the vestibular and cochlear nerves from the brain to the epithelium of the receptor organs. In the organ of Corti the efferent fibers are clearly distinguishable from the afferents and are easily traceable to the inner and outer hair cells. Fibers to the outer rows of hair cells have been observed to course relatively long distances. Each fiber could therefore make synaptic contact with numerous hair cells but this remains to be proven by a more efficacious method of approach such as electron microscope studies of the organ of Corti which has been previously deprived of its efferent innervation. It is certain however, that these fibers are all directly related to the efferent bundle originating in the brain since all traces of acetylcholinesterase activity disappears 7 to 10 days after cutting the bundle in the medulla of the cat and chinchilla.

Additional information has been gained concerning the efferent innervation of the vestibular receptors by means of histochemical studies. Previously Gacek and Rasmussen demonstrated an efferent innervation of all the vestibular receptors with the aid of fiber degeneration methods and Prof. Dohlman demonstrated a high concentration of acetylcholinesterase in some fibers located next to the hair cells of these receptor organs. Recently we have demonstrated the disappearance of the histochemical reaction in all the vestibular receptors following transection of the efferent bundle in the medulla -- the bundle which distributes its fibers to all receptors of the inner ear.

(2) The study of Prof. Dohlman on the morphology and function of the tectorial membrane and cupula is not sufficiently advanced to offer major findings.

(3) Studies on the distribution of fibers arising from the inferior colliculus have disclosed two noteworthy findings.

Part A Project Description (cont'd)

Contrary to previous evidence and prevailing ideas not all of the ascending fibers from the colliculus pass in the brachium to the medial geniculate body nor do all fibers entering the geniculate body terminate in all portions of it. Many fibers leave the brachium of the inferior colliculus to ascend in the reticular formation of both sides to undetermined destinations; other fibers course outside of the principal auditory pathway and terminate in the subthalamic nucleus. Most surprising was the finding that the auditory fibers of the brachium, the principal auditory pathway to the geniculate body, apparently terminate only in the inferior portion of the latter, a portion previously designated as the inferior lobe by Cajal. The dorsal portion or superior lobe (Cajal) remains strikingly free of fiber and synaptic degeneration following destruction of the inferior colliculus. This result has lead us to investigate the nature of the superior lobe under a separate project headed by Dr. Morest.

Significance: Anatomical knowledge of the neuronal relationships existing at the synaptic level between the afferent and efferent auditory system is basic to an understanding of the neuromechanism of hearing. Such information is essential for a foundation upon which to design physiological experiments for testing the functional significance particularly of the descending conduction system.

Proposed course of project: Studies of experimental nature on the efferent innervation of all receptors of the inner ear will be continued and extended to include electron-micrographic studies of experimental material. Anatomical studies of the central auditory system will also be continued. The work of Prof. Dohlman on the mechanism of hair cell stimulation is to be concluded in this laboratory by the end of March 1961.

Part B included: No

Serial No. NINDB-NS-FN-20
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of the afferent extrinsic and intrinsic neuronal connections of the medial geniculate body.

Principal Investigator: Donald Kent Morest

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To locate the origin and course of the afferent projections of the brain stem to the medial geniculate body that are located outside the well known auditory pathways.

(2) To disclose the intrinsic interneuronal connections in the medial geniculate body itself by means of the Golgi methods.

Methods employed: Modified Nauta-Gygax methods for demonstration of axonal and preterminal degeneration subsequent to strategically placed brain lesions in the thalamus and midbrain. When indicated, terminal degeneration in the medial geniculate will be examined in the same brains by means of Glees' or Rasmussen's synapse staining methods. Suitable Golgi methods will be used to elucidate the cellular morphology and intranuclear connections of the medial geniculate.

Major findings: Investigation not sufficiently advanced to offer major findings.

Significance: Detailed information of the connections and cellular organization of the medial geniculate body is necessary to understand fully its role in central auditory function. In particular it is desirable to determine whether the neural input

Part A, Project Description (cont'd)

of the medial geniculate is entirely auditory in nature or whether other sensory modalities may be carried into the auditory system at the thalamic level. A proper conception of the cytoarchitectonics of the medial geniculate is desirable for an appreciation of the organization of the auditory fiber systems and for the evaluation of experimental investigations on the central nervous mechanisms of hearing.

Proposed course of project: To determine the source of the fibers supplying the dorsal portion especially and other portions of the medial geniculate body, other possible sources in addition to the inferior brachium will be investigated and different techniques will be employed simultaneously in several species.

To clarify the cellular organization of the medial geniculate, principal reliance will be on the Golgi methods in mammals and suitable submammalian species.

Part B included: No

SUMMARY REPORT
LABORATORY OF BIOPHYSICS
CALENDAR YEAR 1960

The central objective of the Laboratory of Biophysics continues to be the understanding of the nature and the implications of the ion movements fundamental to the initiation and propagation of the nerve impulse.

The work of the Laboratory during 1960 was the work of the following: John W. Moore, Richard FitzHugh, Robert E Taylor, William J. Adelman, Jr., W. Knox Chandler, Leonard Binstock, Ernest R. Whitcomb, Uichiro Kishimoto and Kenneth S. Cole. During the year Mr. Binstock has come to the Laboratory with an MS in Biophysics and a wide experience in electronics and computing, Mr. Whitcomb has returned to graduate work and Dr. Kishimoto came first as a Rockefeller Fellow and is continuing as a Visiting Associate.

The most impressive numerical measure of the Laboratory work in 1960 is additions to the list of publications. Another ten articles have appeared in scientific journals along with two meeting abstracts. This is to some extent an indication of earlier delays in making work generally available and so should be particularly noted because it may not happen again. But on the other hand this index of Laboratory activity should not show a marked decline during the coming year with four manuscripts already accepted and scheduled for publication.

Although publication is the obligation and so a necessary part of scientific progress it alone is not a sufficient justification for the continuing support or existence of the Laboratory. It is here that the content of work must be taken into account and has to be more a matter of opinion.

Probably in immediate importance and certainly in effort and extent, two articles on the successes and failures of application of the voltage clamp concept to the squid giant axon seem the most significant contributions of the Laboratory. They present this concept in some detail with evidence for the conclusion that, when implemented with care and with a respect for the purely physical problems of stability posed by an axon, the concept is as useful as had been originally assumed. Further they give a considerable basis to expect the anomalies that appear under less than adequate experimental conditions. Subsequent theoretical work on membrane instability and its consequences is now in manuscript and is expected to complete the investigations of the fundamental experimental problems for the near future at least.

In spite of this clarification of the requirements for adequate voltage clamp measurements on the squid axon, the situation is still far from satisfactory. Although powerful and versatile, the electronic control equipment may be both too critical and too slow in its operation for some important problems. A smaller and simpler version is now undergoing an intercontinental field test. Although some progress has been made, two particularly important problems are without indications of their ultimate solution. The first is the measurement and ultimate compensation of the almost certainly present resistance between the potential electrodes and the membrane capacity. Until these steps can be carried out the voltage clamp measurements are inaccurate by an amount that cannot be estimated and in a way that cannot be corrected. The other problem is to develop an effective, reliable and comparatively simple process for the quantitative description of each of the component ionic currents from the results obtained under voltage clamp.

The unique size of the squid axon alone was enough to allow its properties to be by far the best known of any by now, but at the same time it has two serious disadvantages. The first is that it is not widely available and may be also quite unique in other characteristics than size. The other is that, at best, the animal and the axon are rather fragile and the axon is a not particularly reliable or reproducible preparation with quite limited survival. The original and still used technique - with the insertion of a large and long internal electrode - can do nothing but worsen these experimental drawbacks. Early in the development of this Laboratory it was realized that other preparations and other methods were badly needed and the single node and lobster axon were chosen as the most promising. It was consequently a considerable encouragement and relief to find that when applied elsewhere to the single node of medullated axons, the voltage clamp concept was again useful and resulted in membrane characteristics that were better than qualitatively the same as for the squid and without the use of internal electrodes. Considerably more groundwork was done with the lobster axon, and this was followed by somewhat successful attempts to clamp this axon at NMRI. It was possible during this year to join with them in this work on a collaborative project which is now well underway.

With the considerably better basis for confidence in the data upon which Hodgkin and Huxley based their analyses it now becomes possible to turn attentions to the underlying mechanisms and to the implications and applications of them. Along this line, there have been two rather startling developments. The first, now in print, is that the Hodgkin and Huxley empirical expression for the potassium ion current, although reasonably satisfactory for the usual range of axon

phenomena, is quite inadequate to express the delay in the onset of this current after even moderate hyperpolarizations of the axon membrane. The second is that the linear leakage current, that might have represented a constant permeability to the chloride ion, has been found non-linear at times less than 100 microseconds and not to be identified with an inward chloride movement any more than with potassium or sodium. The proposal of a likely mechanism for either of these observations might well be a major breakthrough in the field of membrane permeability.

Another problem of fundamental - not to say crucial - importance is the isotope identification of the principal ion current components which have been attributed to sodium and potassium by Hodgkin and Huxley. This was begun this summer at Woods Hole and is now continuing at Plymouth. The first results are confirmatory but not finally or even satisfactorily so.

An investigation of the effects of a considerable number of cationic, anionic and uncharged detergents on the squid axon membrane has been started. All three types considerably increase the unidentified leakage current but the particularly high effectiveness of the cationic type on all membrane characteristics adds support to the rather usual assumption that this membrane is negatively charged.

Although considerably different in a number of their characteristics the passive metal surfaces are one of the few physical systems with the type of electrical behavior found for the squid axon membrane. An intensive theoretical and experimental investigation for much of the past year at Darmstadt has resulted in a close approach to a complete theoretical description of two of these surfaces.

A joint project with NMRI of more than six years standing is now nearing an end. As part of a search for the molecular mechanisms of enzyme action an extensive series of comparisons was made between the action of synthetic anticholinesterase compounds on the esterase activity and on the effects on whole nerve and single node activity. The compounds cover so wide a spectrum of molecular structure and active group composition and the divergence of their actions on esterase and axon is so great as to practically preclude the possibility that the two processes have anything in common - quite contrary to a widely circulated opinion.

Along with the contributions to publications and at scientific meetings and the invited participations in both, the Laboratory has collaborated extensively and effectively with other scientists with adjacent or overlapping interests. There are two joint projects with NMRI, the earliest with S. L. Friess

and the latest with D. E. Goldman and F. Julian; others with U. F. Franck at Darmstadt, with the Computation Laboratory of the Bureau of Standards, with L. J. Mullins and R. A. Sjodin, then of Purdue at Woods Hole, with J. C. Dalton of Buffalo, with T. Teorell at Uppsala, and with A. L. Hodgkin at Cambridge and Plymouth. In the course of this three members of the staff of the Laboratory have worked as invited guests abroad. Without these ways of cooperation and collaboration science cannot be expected to reach its full potential. Nonetheless this trend should also be a cause for some concern on the part of those responsible for the Laboratory. There have been no similar joint efforts within the NIH and these efforts - that have been so mutually desired and satisfactory - have been broadly but entirely directed at the physical science bases of the life sciences. These observations are submitted by your reporter as evidence that the capabilities of such physical science research in the NIH may be rather less than it will have to have to carry on its mission in any more than a pedestrian way.

The responsible units may find basis for considerable pride in the now emergingly apparent scientific progress made by the staff who are the Laboratory of Biophysics. The mere fact of the publication of twenty basic research papers in scientific literature along with abstracts of six papers given at scientific society meetings in the course of two years at a total personnel cost of less than twenty man-years and in a space of six modules is at least noteworthy. The character and quality of the work should be seriously considered as evidence that fundamental research can and should be done in federal laboratories. There is however in parallel with this written record the very immediate danger that it may come to an early end.

The Laboratory has progressed far past the point at which it should have broadened its staff resources with the complementary personnel and turned more to more direct investigations of the fundamental problems of ionic permeability of membranes. But the last such step, valuable though it was, strained the physical facilities to the utmost. Even without these needed colleagues the effectiveness of each individual on the staff continues to be badly hampered in the available laboratory space and a curtailment of the effectiveness of each has a considerably more deleterious collective effect. More important however is the increasing prospect that in spite of the support in other forms, the dedication to Laboratory objectives and the value of the increasing prestige of the Laboratory, some of the staff may feel required to go elsewhere in the next few years - with a far more than proportional loss to the collective effectiveness. Once a group as new and as small as this loses personnel under such circumstances the process can easily become a cumulative one that would indeed be a poor omen for basic research under these auspices.

Kenneth S. Cole

Serial No. NINDB-B-1
1. Laboratory of Biophysics
2. - - - - - - - - - -
3. Bethesda, Maryland
4. Continuation of 1959 project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses

Principal Investigators: Adelman, W. J. Jr., Binstock,L., and Kishimoto, U.

Other Investigators: Taylor, R. E, Moore, J. W., and Cole, K. S.

Cooperating Unit: Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years:
Total: 5.2
Professional: 4.4
Other: .8

Project Description:

Objectives: The ionic current flow across the membrane of the squid giant axon has been measured, without the complications of excitation and propagation, after a sudden change of the membrane potential. The currents have been analysed in terms of the membrane permeability to sodium and potassium ions. Much of classical nerve physiology is explained by these permeabilities which are themselves not understood. The long range objectives are the further interpretation of nerve function in terms of these fast ionic permeabilities and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: The original instrumentation of the potential control concept has been extensively modified and expanded to improve its accuracy, power, speed and versatility. The membrane potential is now measured between micropipette

internal and nearby external reversible electrodes and is maintained and changed as required by an electronic control system. This system computes, produces and measures the necessary flow of membrane current between axial and external axon electrodes. These experimental methods have been described in moderate detail and are in process of publication (Part B). A manuscript covering the design of the microelectrode preamplifier has been completed and some progress has been made in the external and axial current electrode performance and in the axon chamber design.

Major Findings: The work from previous years on adequate and inadequate implementation of the voltage clamp concept was completed as far as possible and is now published (Part B). A considerable extension of the original empirical description of the potassium ion current was also published (Part B). Manuscripts have been finished on the measurement of the internal sodium ion concentration by means of the membrane sodium potential and on determinations of the effect of external divalent ions on the sodium ion flux by this method.

The leakage current which appears in addition to the larger, relatively slow, delayed rectification currents of sodium and potassium ions under voltage clamp conditions was investigated in some detail. As previously suspected, it also shows a rectification characteristic but with a time constant of less than 100 microseconds. It further appears not to be an inward movement of sodium, potassium or chloride. A report of these results has been submitted for publication.

Experiments on the effects of detergents on membrane properties showed that cationic, anionic and non-ionic detergents have different effects on the membrane. While all such detergents increase the aforementioned leakage current, cationic detergents are the more powerful followed by anionic and finally by the non-ionic. Only cationic detergents irreversibly reduce both the resting and the action potentials. Extreme differences among the effective concentration thresholds for the three types of detergents suggest that the membrane is negatively charged.

Bridge techniques were developed to measure the high frequency alternating current impedance of the squid axon membrane. A pilot study was begun and only preliminary results have been obtained so far. These results are being analyzed to guide the design of experiments for next summer. An attempt to measure the membrane series resistance was dependent upon the success of the bridge measurements and the preliminary data are still undergoing analysis.

A development from this project is another attempt to extend the work of the Laboratory and the application of the voltage clamp to membranes other than that of the squid axon. This is a new collaborative project with NMRI which is reported as NINDB-B-6.

A new development is the long-needed direct comparison of voltage clamp currents with tracer isotope fluxes which was initiated this year in collaboration with Purdue University, the University of Cambridge, and the Plymouth Laboratory and is reported as new Project NINDB-B-7.

Scientific Significance: It seems probable that the considerable amount of work on the squid axon which has been published during the year will be a rather significant contribution towards the understanding not only of this membrane but also those of other cells and of membrane processes in general. Evidence has been presented from which it is concluded that the original experimental basis for the revolutionary Hodgkin-Huxley development is qualitatively sound and a considerable progress has been made towards the establishment of necessary and sufficient criteria for usefulness of future work based upon this still unique approach.

Proposed Course of Project: The analysis of data obtained from the 1960 experimental season has not progressed far enough to give a basis for planning continuation of work already started or the initiation of new approaches beyond those already mentioned.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cole, K. S., and Moore, J. W. Ionic current measurements in the squid giant axon membrane. J. Gen. Physiol. 44: 123-167, 1960.

Cole, K. S., and Moore, J. W. Potassium ion current in the squid giant axon: Dynamic characteristic. Biophysi. J. 1: 1-14, 1960.

Moore, J. W. Operational Amplifiers. Physi. Tech. in Bio. Research, Vol. 6, W. L. Nastuk, Ed., Academic Press, Inc., 1960. (in press)

Moore, J. W., and Cole, K. S. Voltage Clamp Techniques. Physi. Tech. in Bio. Research, Vol. 6, W. L. Nastuk, Ed., Academic Press, Inc., New York, 1960. (In press)

Schwan, H. P., and Cole, K. S. Bioelectricity: Alternating current admittance of cells and tissues. Med. Physics, Vol. 3, Chicago, Ill. Otto Glasser, Ed., Year Book Publishers, pp. 52-56, 1960.

Taylor, R. E, Moore, J. W., and Cole, K. S. Analysis of certain errors in squid axon voltage clamp measurements. Biophysi. J. 1: 161-202, 1960.

Honors:

K. S. Cole was elected a member of the Editorial Board of the new Biophysical Journal. He also visited the University of Uppsala as Distinguished Research Professor.

J. W. Moore was appointed a member of the Editorial Board of the Review of Scientific Instruments.

Serial No. NINDB-B-2
1. Laboratory of Biophysics
2. - - - - - - - - - -
3. Bethesda, Maryland
4. Continuation of 1959 project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations

Principal Investigators: FitzHugh, R., and Chandler, W. K.

Other Investigators: Taylor, R. E; U. F. Franck(Darmstadt)

Cooperating Units: Computation Laboratories, National Bureau of Standards and Division of Research Services, NIH, Technische Hochschule, Darmstadt.

Man Years:

Total:	3.1
Professional:	2.5
Other:	.6

Project Description:

Objectives: To investigate the bases, consequences, and extensions of ionic permeability concepts by mathematical analysis and computation.

Methods Employed: Mathematical analysis, with particular emphasis on theories of nonlinear differential equations, stability, and cable equations, has been used in conjunction with the electronic analog computer in this laboratory and the digital computers at the NIH and at the National Bureau of Standards.

Major Findings: The analysis of the properties of the Hodgkin-Huxley equations, using phase plane concepts and the Berkeley analog computer has been continued. Some of the general approaches and their applications to the study of threshold excitation and the production of plateau action potentials have been published (Part B). Modifications of the equations which reproduce data on the lobster axon are also in print (Part B).

The theory of the submarine cable has long been the only satisfactory physical basis for the quantitative description of nerve behavior. A history of this development with its modern applications and limitations has been assembled for the first time and will appear as a monograph chapter (Part B).

Digital computations in which the Hodgkin-Huxley equations have been combined with cable theory have shown that as the linear leakage conductance is increased, the velocity of propagation decreases. For leakage conductances greater than 8.9 mmho/cm.^{2} a propagated action potential is impossible. The curve relating conduction velocity to leakage shows two velocities for each leakage conductance, one of which is probably unstable.

An analysis of the Hodgkin-Huxley equations which have been linearized about an operating point has been useful in investigating the stability characteristics of a nerve during a voltage clamp pulse. As the Nyquist criterion provides a convenient means for determining the border between stability and instability, the digital computer at the NIH has been programmed to compute the alternating current admittance of the linearized model. The results obtained indicate that the uniform nerve is stable for all depolarizing pulses if the resistance of the axial wire, axoplasm, sea water, and external electrodes is below 19 ohm cm.^{2} for a depolarization from the resting potential or below 12 ohm cm.^{2} for a nerve previously hyperpolarized 20 mv. The maximum amount of possible instability appears at a depolarization of 30 mv. which corresponds to the inflection point in the negative resistance region of the peak inward current-voltage curve. These exact calculations support earlier intuitive notions that the stability characteristics for nerve are not much different from those of a system having a steady state voltage characteristic similar to the peak inward current versus voltage curve. A manuscript is in preparation.

The application of cable theory to this problem has suggested that a smaller length of nerve is inherently more stable than a longer length, if suitable boundary conditions are used. In fact, to

the extent that the guard electrodes preserve this boundary stability, the theory indicates that a small length is a prerequisite to adequate voltage control in a powerful nerve. This is a result of using the peak inward current-voltage characteristic of experimental nerves as a steady state curve, an assumption which was shown to be reasonable for the Hodgkin-Huxley model. A manuscript is in preparation.

Since March, 1960, Dr. FitzHugh has been working in the Department of Electrochemistry of the Technische Hochschule of Darmstadt, Germany, with Professor Ulrich F. Franck, on mathematical models of passivable iron electrodes. Differential equations have been obtained for an electrode in H_2SO_4, which reproduces the stable current oscillations obtained experimentally under voltage clamp. This model has two variables of state, the fraction (α) of the surface area covered by the oxide film, and the pH at the electrode surface, which interacts with the oxidation reaction. Analog computations of these equations have been carried out, and two papers describing this work are being written.

Analysis of equations for the Ostwald-Lillie model of nerve (Fe in HNO_3) has been begun. This model has 3 variables of state: α as above, the electrode potential, and the HNO_2 concentration at the electrode surface. HNO_2 enters into an auto-catalytic reaction which contributes a current component having a dynatron characteristic. This is expected to produce nervelike behavior when the electrode is subjected to current clamp. pH changes have only a secondorder effect here and have been neglected for the time being.

Dr. FitzHugh has also visited the laboratories of Hodgkin, Huxley, and Barlow in Cambridge, England, Hutter in London, Sten-Knudsen in Copenhagen, Teorell in Uppsala, and Frankenhaeuser and Skoglund in Stockholm, for varying periods, for discussion of theoretical problems in excitation. Seminars on the mathematical analysis of models of excitation, including the Hodgkin-Huxley model of nerve, were given in Cambridge and Uppsala.

Scientific Significance: The concept and the measurements of the sodium and potassium ion movements across the squid axon membrane offer a highly specific, and general approach to the factors underlying normal and pathological nervous processes which cannot as yet be investigated directly in higher animals and man. An analysis of the mathematical properties of the Hodgkin-Huxley equations makes possible a better understanding of their possible modifications and their limitations, than is obtainable by a purely physical interpretation.

Proposed Course of Project: The results of the work on stability will be extended and put into a form suitable for publication. The stability properties associated with an active cable are currently being investigated further and it is expected that this work will continue.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cole, K. S. Non-linear current-potential relations in an axon membrane. J. Gen. Physiol. 1961. (accepted)

Dalton, J. D., and FitzHugh, R. Applicability of Hodgkin-Huxley Model to experimental data from the giant axon of lobster. Science 131: 1533-1534, 1960.

FitzHugh , R. Thresholds and plateaus in the Hodgkin-Huxley nerve equations. J. Gen. Physiol. 43: 867-896, 1960.

Taylor, R. E . Cable Theory. Physi. Tech. in Bio. Research, Vol. 6, W. L. Nastuk, Ed., Academic Press, Inc., 1960. (in press)

Serial No. NINDB-B-3
1. Laboratory of Biophysics
2. - - - - - -- - - -- -
3. Bethesda, Maryland
4. Continuation of 1959 project

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Acetylcholinesterase Inhibition and Nerve Activity

Principal Investigators: Whitcomb, E.R.

Other Investigators: Friess, S.L. (NMRI)

Cooperating Unit: Naval Medical Research Institute

Man Years:
Total: 1.0
Professional: 0.8
Other: 0.2

Project Description:

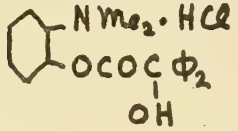
Objectives: To determine the effect of sterospecifically tailored aminoalcohol derivatives on the electrical activity of the single node, in terms of threshold and action current parameters.

To study mechanisms of action at the molecular level including the strength and nature of drug-tissue interaction.

Methods Employed: A single frog sciatic nerve fiber is isolated and mounted for recording from a single node. The node is stimulated directly once per second. The time course and amplitude of the threshold and action current of the node are studied as functions of concentration and molecular structure of the blocking compounds added to the Ringer's bathing medium.

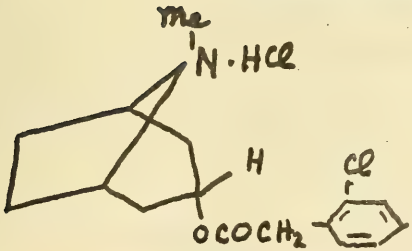
Major Findings:
1. cis-tertiary benzilate (cis-2-dimethylaminocyclohexyl benzilate) trans-tertiary benzilate

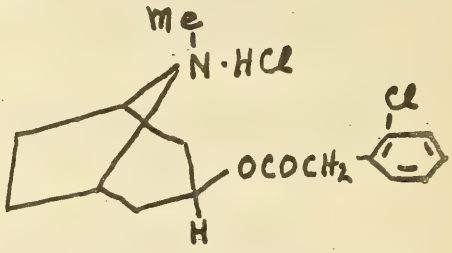
(trans-2-dimethylamincocyclohexyl benzilate)



The cis-member is three times more effective than its trans-counterpart in attenuating the action current and increasing the threshold. However, the trans-produced a greater degree of irreversibility as indicated by the amount of recovery of the action and threshold upon replacing the drug media with Ringer's solution.

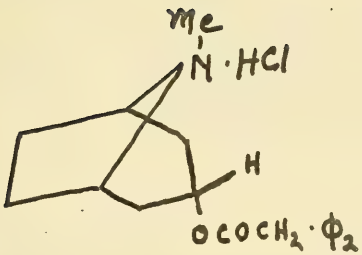
2. tropine Θ-chlorophenylacetate ψ-tropine Θ-chlorophenylacetate

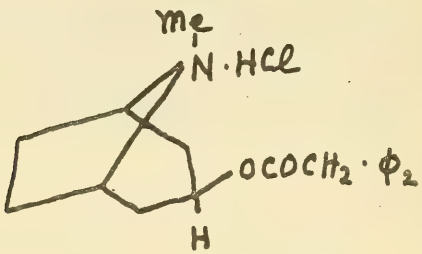




The tropine Θ-was five times more effective than ψ-tropine in attenuating the action current and increasing the threshold. Also the tropine Θ produced the greater degree of irreversibility.

3. tropine diphenylacetate ψ-tropine diphenylacetate





ψ tropine diphenylacetate was five times more effective than tropine diphenylacetate in attenuating the action current and increasing the threshold. Both produced approximately the same degree of irreversibility.

4. The order of effectiveness of the group is as follows:

cis-tertiary benzilate > ψ tropine diphenylacetate > trans-tertiary benzilate > tropine Θ chlorphenylacetate > tropine diphenylacetate > ψ tropine Θ chlorophenylacetate.

5. These six compounds, along with those previously reported, essentially covers the complete range of molecular parameters. As with the others these have also been investigated for their in vitro enzyme inhibition in a parallel project.

The comparison between these inhibitions and the node blockades for the complete series lead only to the conclusion that the two processes have nothing in common.

Scientific Significance: These studies aid in evaluating the role of acetylcholinesterase in nerve function and in formulating the conditions characteristic of one type of nerve impulse blockade.

Proposed Course of Project: This project will be held in abeyance until further work is done on the "voltage clamp" for the single frog node.

The comparison between enzyme inhibition and nerve activity has now been completed and the final results are being prepared for publication to complete this project. NMRI now plans to turn to this mechanisms of node blockade by a voltage clamp investigation on its own responsibility.

Part B included - yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project

Whitcomb, E.R., and Friess, S.L. Blockade of the Action Current In Single Nodes of Ranvier from Frog Nerve by Physostigmine and Certain Aminoalcohols Derivatives. Arch. Biochem. and Biophysics 90:260-270, 1960

Serial No. NINDB-B-6
1. Laboratory of Biophysics
2. -- - - - - - —
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Ionic Permeabilities of Nerve Membranes: Investigation of new methods and of other axons than squid.

Principal Investigators: Moore, J.W., Julian, F. (NMRI), Goldman, D.E. (NMRI)

Other Investigators: None

Cooperating Unit: Naval Medical Research Institute

Man Years:
Total: .4
Professional: .3
Other: .1

Project Description:

Objectives: 1. To develop methods for measuring individual ion permeabilities of non-myelinated axons which are simpler and faster than those conventionally used.

2. To study ionic conductances in the lobster giant axon.

3. To study the interaction of electrical and mechanical properties of axons.

Methods Employed: Use of electronic feedback and/or sucrose gaps to minimize external leakage and thereby provide an accurate measure of membrane potentials without the usual requirements of internal electrodes. Potential control of the membrane with current fed longitudinally appears feasible. Computations on potential and conductivity data can provide information on specific ion permeabilities and currents during an action potential. Simple

analog computer elements are now fast enough to do this in real time, i.e., operate directly on the original data.

Major Findings: Sucrose insulation gaps used with the giant axon of the lobster have been found very effective for obtaining membrane potential and current data.

Scientific Significance: Since the Hodgkin-Huxley ionic conductance equations were formulated for the squid nerve membrane, there has been an increasing appreciation among electrophysiologists of the need to interpret more classical data (such as changes in action potential shape or propagation velocity) in these terms. The development of a voltage clamp technique simple enough for widespread use would be of considerable benefit.

Alternatively the development of simple methods to obtain specific ion currents and conductances during propagated action potentials should also advance the rate at which an understanding of membrane phenomena occurs. While data from such experiments would be less complete than obtained from the voltage clamp, the experimental set up would be far less exacting and could be widely employed.

An understanding of the mechano-electrical behavior of axons may aid in the elucidation of the activity of mechanoreceptors.

Proposed Course of Project: To continue the development of membrane potential recording for non-myelinated axons without the use of internal electrodes.

To pursue the development of voltage clamp and mechanical deformation techniques using the sucrose gap.

To develop a method of computing specific ionic current and conductance patterns during propagated action potentials.

Part B - None

Serial No. NINDB-B-7
1. Laboratory of Biophysics
2. - - - - - - - -- - -
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Ionic Permeabilities of Squid Giant Axon Membrane. Tracer and Electrical Experiments and Analyses.

Principal Investigators: Adelman, Jr., W.J., Mullins, L.J., (Purdue) Sjodin, R.A., (Purdue) Hodgkin, A.L., (Cambridge)

Other Investigators: Binstock, L., Cole, K.S.

Cooperating Units: Purdue University, University of Cambridge, Marine Biological Laboratory Plymouth Marine Station

Man Years:
Total: .5
Professional: .4
Other: .1

Project Description:

Objectives: The ionic current across the membrane of the squid giant axon has been measured, without the complications of excitation and propogation, after a sudden charge of the membrane potential. These currents are to be compared with the fluxes of tracer isotopes to test the validity and to extend the application of their analyses in terms of the membrane permeability to sodium and potassium ions and to test the validity of the present interpretations of isotope flux measurements.

Methods Employed: The voltage clamp instrumentation is essentially the same as developed and used under Project NINDB-B-1. A simpler and more

portable version of the electronic control system was designed and tested, and has been taken on a field trip to Plymouth. The isotope procedures have so far been rather orthodox although undergoing a continual modification.

Major Findings: The initial experiments were carried out at Woods Hole with Drs. Mullins and Sjodin. Membrane current components under voltage clamp were compared with outfluxes of radioactive sodium and potassium ions. Preliminary results show that the large current components exhibiting delayed rectification, which have been described by Hodgkin and Huxley as sodium and potassium currents, can be accounted for by tracers within 15 percent for sodium and 30 percent for potassium

Radioactive sodium loaded axons were pulsed for short durations and low frequencies to values of the membrane potential which resulted in an outward early membrane current. The corresponding sodium efflux was determined by collecting the effluent radioactive sodium into a sodium "free" external sea water maintained rapidly circulating past the axon.

In a similar manner radioactive potassium loaded axons were pulsed with longer duration pulses at low frequencies and the membrane outward current supposedly due to potassium movement was compared with the potassium efflux. The correspondence between charge and efflux was less than predicted and further experiments are planned.

Further analyses of the data at higher frequencies and higher external sodium ion concentrations are under way.

The principal NIH investigator has taken the essential axon and control equipment to England to continue the project in collaboration with Professor Hodgkin at Cambridge and at Plymouth for the current squid season.

Scientific Significance: The preliminary results suggest that more, more satisfactory, and more complete experiments will provide independent evidence to so adequately support the Hodgkin-Huxley interpretations of voltage clamp data as to practically preclude the possibility that they are not correct. If this is indeed the case it will mark the completion of an era of radical reorientation and lay a firm foundation for future progress in the understanding of all ionic membrane phenomena.

Proposed Course of Project: As time and facilities permit the work will be extended to include influx as well as further outflux experiments and the inclusion of other pertinent variables. Such work may be expected to provide a firmer basis for the determination of separate ion permeabilities, a test of the present concept that these permeabilities are purely dissipative parameters, and a further examination of the significance of tracer ion fluxes.

Part B- None

LABORATORY OF NEUROCHEMISTRY

SUMMARY, 1960

The investigation on the biosynthesis of complex lipids was continued. A study on the biosynthesis of cerebrosides in patients afflicted with Gaucher's disease or with Niemann-Pick disease was completed. It was found that in Gaucher's disease, the rate of cerebroside synthesis in splenic tissue was not sufficiently elevated to warrant the conclusion that the disease was due to an accelerated synthesis of this lipid. The interconversion of glucose and galactose in these tissues also appeared to be normal, and it was suggested that further investigations be directed towards the elucidation of the catabolic pathways of cerebrosides.

The *in vivo* synthesis of the N-acyl bond in the ceramide portion of sphingolipids is as yet poorly understood, and efforts were directed towards the development of methods which allowed the investigation of the N-acylation of sphingosine in brain. The fatty acid portion in some of the sphingolipids is represented by fatty acids which have a chain length of more than 18 carbons. The origin of these long-chain fatty acids is unknown. Thus, an investigation of the origin and activation of the long chain fatty acids ($C \geq 20$) seemed to fit well into an already established program on the biosynthesis of fatty acids.

Original studies in this laboratory, that malonyl coenzyme A was one of the key intermediates in fatty acid synthesis, had been confirmed by several other investigators. A method was devised for the chemical synthesis of carbon labeled malonyl Coenzyme A. This and other labeled precursors were utilized in the further study of the reaction mechanism leading to the formation of palmitic or stearic acids. Various observations on the requirements of the fatty acid synthesizing systems indicated that the enzymes involved were of the sulfhydryl-type and that juxtaposed R-SH groups within the enzyme were an integral part of its active site. It was demonstrated that the synthesis of palmitic acid is accomplished by the condensation of one mole of acetyl coenzyme A with seven moles of malonyl coenzyme A. A search for possible intermediates in the elongation of the carbon chain failed to yield any products which could serve as acceptors for malonyl CoA, and further investigation indicated that the long chain fatty acid was formed directly on the enzyme surface without liberation of intermediary products. The reductive steps involved were studied with the use of tritium labeled triphosphopyridine nucleotide, and the observation of the incorporation of tritium into the beta-positions only of the formed palmitate was considered a significant finding and thought to support the mechanism of fatty acid synthesis postulated in this laboratory.

The carboxylation of acetyl coenzyme A to malonyl coenzyme A was further investigated. The enzymes responsible for this carboxylation reaction were further purified even though no clean separation of the propionyl from the acetyl CoA carboxylase was achieved. This particular reaction was studied in some detail, and the metal and cofactor requirements for this reaction were established.

The condensation of 5-carbon fragments to form cholesterol was further studied, and the pathways established for yeast were confirmed in the animal. A chemical resemblance of the isopentenyl pyrophosphate isomerase enzyme-substrate compound and penicillin was found. This suggested a possible analogy between penicillin and terpene biosynthesis.

Techniques for the chemical synthesis of certain liponucleotides previously discovered in this laboratory were elaborated and utilized in an investigation of the possible role of the inositol phosphatides in the mammalian cell. The enzymatic synthesis of a number of structural phosphatidyl inositols was investigated, and attempts were made to identify the various inositol containing phospholipids.

The structure and function of the brain gangliosides were investigated in collaboration with Drs. R. A. Resnik and R. L. Irwin from this Institute. Structure-function relationship studies indicated that the terminal neuraminic acids in gangliosides appears to be of great importance in the function and the properties of this complex glycolipid. It was discovered that gangliosides were not large polymers but were micellar aggregates in aqueous solutions. A method was devised for the isolation of highly purified gangliosides. Chemical studies have shown that even the highly purified material was composed of different sub-units, and no simple repeating unit for the high molecular aggregate could be postulated. It was further observed that the gangliosides seem to be able to antagonize the effects of some quarternary ammonium compounds. The possible role of these and related materials as cell receptor sites was considered, and studies initiated to elucidate their mechanism of action.

In a continuation of our general studies on the synthesis of enzymes, the effects of some carcinogenic hydrocarbons were observed on the activity of a number of liver enzymes. It was found that the activity of certain microsomal enzymes seemed to decrease in the course of chronic administration of these hydrocarbons while other enzymes seemed to respond with increased activity in what appeared to be a compensatory mechanism. Similar observations were made following intraperitoneal injection of some cyclic hydrocarbons. The synthesis of a number of microsomal enzymes was investigated under these conditions. The mechanism by which these remarkable enzyme inductions take place will require further study.

In an effort to determine whether or not redox reactions participated in excitation and impulse conduction, the effect of coenzyme Q and derivatives thereof were investigated on chronically stimulated unmyelinated nerve fibers.

Serial No. NINDB-NC-1
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biosynthesis of Sphingolipids

Principal Investigators: Roscoe O. Brady and Eberhard G. Trams

Other Investigators: Joseph D. Robinson

Cooperating Units: None

Man Years
Total: 1.6
Professional: 0.6
Other: 1.0

Project Description

Project: Investigation of the metabolism of complex cerebral lipids.

Objectives: To elucidate the pathway of formation of sphingolipids and to determine the rate of turnover and metabolic fate of these substances.

Methods Employed: The synthesis of gluco- and galacto-cerebrosides was investigated with the aid of isotopic precursors. The formation of the glycosidic linkage, the formation of the N-acyl linkage of sphingosine, and various possibilities for the formation of the long chain fatty acids in the ceramide portion of cerebrosides were studied with conventional analytical techniques.

Major Findings: The investigation of cerebroside synthesis in spleens of patients with Gaucher's disease or with Niemann-Pick disease was completed. In situ formation of cerebrosides in spleens was demonstrated. It was concluded that the rate of cerebroside formation in Gaucher's disease was not sufficiently elevated to warrant the conclusion that the biochemical lesion in this particular type of lipodystrophic disease was due to an increased rate in lipid synthesis.

Part A, continued:

Limited progress was made in establishing the mode of ceramide formation in sphingolipids. Net synthesis in the systems investigated appeared to be rather low.

Scientific Significance: The sphingolipids constitute a major portion of the lipids of the central nervous system (CNS) and of the myelin sheath, and research dealing with the formation of these compounds might lead to an understanding of the basic lesions in the lipodystrophic diseases or the demyelinating diseases. The source and the utilization of the fatty acids with a chain length exceeding 18 carbons is of fundamental interest. Since these acids are the major component of the ceramide portion of the sphingolipids, it is of great interest to learn more about their mode of formation and activation.

Proposed Course of Project: It is proposed to continue investigations on the formation of the cerebrosides and the ceramides. Since the studies of the cerebroside synthesis in Gaucher's disease revealed essentially normal rates of synthesis, it is proposed to investigate the catabolism of these lipids in patients afflicted by the disease.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Trams, E. G. and Brady, R. O. Cerebroside synthesis in Gaucher's disease. J. Clin. Inves., 1960, 39: 1546-1550.

2. Brady, R. O. Enzymatic Aspects of Neurological and Mental Diseases in Enzymes in Health and Disease, pp. 141-157, C. C. Thomas Publisher, Chicago, 1960.

Serial No. NINDB-NC-6
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biosynthesis of Aromatic Compounds

Principal Investigator: Roscoe O. Brady

Other Investigators: W. Siegelman, M. D. Lane

Cooperating Units: Plant Physiology Laboratory, Pioneering Research Group, U. S. Department of Agriculture, Beltsville, Md. and Department of Biochemistry, Virginia Polytechnic Institute, Blacksburg, Va.

Man Years:
Total: 0.1
Professional: 0.1
Other: 0

Project Description

Project: Investigation of the mechanism of formation of aromatic compounds.

Objectives: To discover the enzymatic pathway of formation of methylsalicylate which arises apparently through the condensation of 2-carbon fragments derived from acetyl-CoA.

Methods Employed: The enzymatic synthesis of methylsalicylate was investigated, using standard enzymological methods as well as radioactive tracer techniques.

Major Findings: It has been ascertained that labeled acetate is a preferential precursor for the biological formation of compounds containing benzene rings. There exists, particularly in plant tissue, a biological route for benzene ring formation other than via sedoheptulose, quinic, shikimic acids, etc. We have established the preferential conversion of labeled acetate to 6-methyl salicylic acid *in vitro*.

Part A, continued:

Scientific Significance: The mechanism of condensation of four 2-carbon metabolically active fragments with attendant cyclization and desaturation represents a completely unresolved biological phenomenon. Insight into the nature of this process may shed light on the pathway of formation of important quinones such as vitamin K and coenzyme Q.

Proposed Course of Project: The project will be discontinued pending Dr. Brady's return from California.

Part B included: No

Serial No. NINDB-NC-7
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Metabolism of Inositol

Principal Investigator: B. W. Agranoff

Other Investigators: H. Eagle

Cooperating Units: Laboratory of Cell Biology, NIAID

Man Years:
Total: 1.1
Professional: 0.5
Other: 0.6

Project Description:

Project: Investigation of the function of inositol as a vitamin.

Objectives: To determine the biochemical site of the inositol required by cells in culture.

Methods Employed: Inositol, labeled with radioactive tracers, was incubated in the presence of cells grown in culture and the radioactive products analyzed for inositol content and for radioactivity. The inositol concentration was determined by both microbiological and chemical methods. A special flask consisting of two culture vessels separated by a dialysis membrane was used to ascertain whether different cell species could supply dialyzable factors to one another.

Major Findings: Inositol-dependent mammalian cell types appear to require inositol for the production of structural phosphatidyl inositol. Inositol was also found in a bound form in protein and nucleic acid fractions. In addition, certain cell types were able to synthesize inositol to support growth in deficient cell types.

Part A, continued:

Scientific Significance: While it has been previously shown that certain vitamins or cofactors may be produced by intestinal bacteria, the present study indicates that particular somatic cell types may possibly furnish constituents for others. Techniques for the chemical synthesis of liponucleotides previously discovered in this laboratory have been developed. The enzymatic reactions of this new class of compounds should prove important in understanding the chemical function of cell membranes.

Proposed Course of Project: This project has been discontinued.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Eagle, H., Agranoff, B. W., and Snell, E. E. The biosynthesis of meso-inositol by cultured mammalian cells and the parabiotic growth of inositol-dependent and independent strains. J. Biol. Chem., 1960, 235: 1891-1893.

2. Agranoff, B. W. Cytidine nucleotides, pp. 38-50. Chapter in: Neurochemistry of nucleotides and amino acids, Brady, R. O. and Tower, D. B., eds. New York, John Wiley and Sons, Inc., 1960, 292 pp.

Serial No. NINDB-NC-14
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Enzymatic Synthesis of Fatty Acids

Principal Investigator: Roscoe O. Brady

Other Investigators: E. G. Trams

Cooperating Units: None

Man Years
Total: 2.0
Professional: 0.8
Other: 1.2

Project Description:

Project: Investigation of the mechanism of the formation of long chain fatty acids.

Objectives: The key intermediate in the synthesis of long chain fatty acids was shown to be malonyl coenzyme A. The project at present is concerned with the enzymatic mechanism responsible for the condensation reaction between malonyl CoA and an acyl-CoA acceptor. Successive condensations and reductions lead to the synthesis of palmitic or stearic acid. Objectives of this study are the identification of the various steps, the intermediates formed and the enzymed involved.

Methods Employed: Carbon- or tritium-labeled precursors were used as substrates for rat brain and rat liver enzymes. Gas chromatographic analyses were performed for the identification of the reaction products. Conventional enzyme fractionation procedures were employed to prepare the requisite enzymes.

Major Findings: It was demonstrated that a soluble enzyme obtained from liver or brain catalyzed the condensation of 1 Mole of acetyl CoA with 7 Moles of Malonyl CoA to form palmitic acid. Fatty acid synthesizing enzyme obtained from brain was found to be devoid of malonyl CoA decarboxylase and thus appeared well suited for the study of

Part A, continued:

the intermediates involved in the synthesis of palmitate. All the evidence obtained pointed to the fact that no free intermediates were formed during the condensation process and that the condensation reactions as well as the subsequent reductions occurred without release of the reactants from the enzyme surface. A study of the involved enzymes showed that a key position is held by vicinal sulfhydryl groups and a mechanism for the synthesis of long chain fatty acids was postulated.

Scientific Significance: The demonstration that malonyl CoA is the active intermediate in the process of fatty acid synthesis constitutes a finding of considerable importance for understanding the synthesis of lipids, whose participation is required in so many varied biological phenomena. It is obviously quite improbable that fatty acid synthesis occurs to a significant extent via the reverse of the process of fatty acid oxidation. Hence, the elucidation of the explicit details of the mechanism of fatty acid synthesis is of paramount interest.

Proposed Course of Project: Since the mechanism for the synthesis of fatty acids containing up to 18 carbons appears now established, an attempt will be made to extend the investigation to the synthesis of fatty acids containing up to 24 carbons. Preliminary studies have shown that these acids are not necessarily synthesized by the same mechanisms, and it is proposed to investigate the mechanism of the elongation of the carbon chain beyond the 18th carbon.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Brady, R. O. and Tower, D. B., eds. Neurochemistry of nucleotides and amino acids. New York, John Wiley and Sons, Inc.m 1960m 292 pp.

2. Brady, R. O., Trams, E. G., and Bradley, R. M. Participation of closely juxtaposed sulfhydryl groups in fatty acid biosynthesis. Biochem. and Biophys. Research Communications, 2: 256-258, 1960.

3. Trams, E. G. and Brady, R. O. The synthesis of malonyl-C^{14} coenzyme A. J. Am. Chem. Soc., 82: 2972, 1960.

4. Brady, R. O., Bradley, R. M., and Trams, E. G. Biosynthesis of fatty acids I. studies with enzymes obtained from liver. J. Biol. Chem., in press.

5. Brady, R. O. Biosynthesis of fatty acids II. Studies with enzymes obtained from brain. J. Biol. Chem., in press.

Serial No. NINDB-NC-15
1. Neurochemistry
2. Lipid Chemistry
3. Munich, Germany

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biosynthesis of Cholesterol

Principal Investigator: B. W. Agranoff

Other Investigators: None

Cooperating Units: Max Planck Institut für Zellchemie, Munich, Germany.

Man Years
Total: 1.1
Professional: 0.5
Other: 0.6

Project Description:

Project: To elucidate the biochemical pathways for the formation of cholesterol and related substances.

Objectives: To isolate intermediate substances between isopentenyl pyrophosphate and squalene in the pathway to cholesterol formation.

Methods Employed: C^{14}-labeled precursors were incubated with tissue preparations and reaction products were isolated and identified.

Major Findings: Pathways elucidated in yeast were confirmed in animal tissues. The condensing system to make a 30 carbon chain from six 5-carbon precursors was termed "Terpene Synthetase" to emphasize the polymeric nature of the product as well as the irreversibility of the reaction. A chemical resemblance of the isopentenyl pyrophosphate isomerase enzyme-substrate compound and penicillin was found, suggesting an analogous relationship between terpene formation and the formation of penicillin in micro-organisms.

Part A, continued:

Scientific Significance: The pathways established by work done in collaboration with Professor Lynen's laboratory in Munich clearly establishes the mechanism of cholesterol formation in organisms which are able to form this sterol. The large amount of cholesterol in brain and its low metabolic turnover coupled with the fact that it is largely unesterified in brain indicate a structural function for brain cholesterol and an important role for this synthesis pathway.

Proposed Course of Project: This project is completed.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Agranoff, B. W., Eggerer, H., Henning, U., and Lynen, F. Biosynthesis of terpenes. J. Biol. Chem., 235: 326-332, 1960.

Serial No. NINDB-NC-16
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Role of Redox Reactions in Excitation

Principal Investigators: Roscoe O. Brady and Constantine Spyropoulos

Other Investigators: None

Cooperating Units: Section on Special Senses, Laboratory of Neurophysiology, NINDB

Man Years
Total: 0.1
Professional: 0.1
Other: 0

Project Description:

Project: To investigate the involvement of biological oxidation-reduction systems in excitable tissues.

Objectives: To determine if redox systems participate in the phenomena of excitation and impulse conduction.

Methods Employed: Chronically stimulated unmyelinated nerve fibers will be examined for the ratio of oxidized and reduced components of known biological oxidation-reduction systems.

Major Findings: The report (Spyropoulos, C. S. and Brady, R. O., Science, 129: 1366, 1959) that oxidating agents are capable of causing a decrease of the resting potential of a nerve fiber with concomitant spontaneous continuous firing of impulses suggests that the membrane of viable nerve tissue normally contains a component in the reduced state. Investigations were extended to the study of the role of coenzyme Q as a possible mediator in pulse propagation.

Part A, continued

Scientific Significance: The intimate details of the phenomenon of biological excitation remain to be elucidated. Conclusive demonstrations of the nature and function of the reactants in this process is of primary importance.

Proposed Course of Project: The project will be discontinued pending Dr. Brady's return from California.

Part B included: No

Serial No. NINDB-NC-17
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Structure and the Biosynthesis of Brain Gangliosides

Principal Investigator: Eberhard G. Trams

Other Investigators: None

Cooperating Units: None

Man Years
Total: 1.6
Professional: 0.8
Other: 0.8

Project Description:

Project: Investigation of the chemical structure and the biosynthesis of brain gangliosides.

Objectives: To investigate the identity of the components and their arrangement within this apparent high molecular polymer and to establish the biosynthetic pathways which lead to the assembly of this complex structure.

Methods Employed: A new method was developed for the isolation and purification of gangliosides from brain. Highly purified preparations were analyzed by standard procedures for their component building blocks.

Major Findings: In agreement with findings of Klenk et al., it was established that the glycolipid was composed of sphingosine, fatty acids, glucose, galactose, N-acetyl galactosamine and N-acetyl neuraminic acid. Quantitative analysis of the components indicated that brain ganglioside was a mixture of various very similar compounds and that no simple repeating unit could be postulated. Sedimentation studies indicated further that the polymer was not bound through co-valent linkages, but that it was an aggregate in aqueous solutions. Enzymatic degradation indicated that materials could be obtained which resembled

Part A, continued

in many ways the so-called cytosides. Studies on the incorporation of labeled precursors into ganglioside showed that total synthesis and turnover of the material is very slow.

Scientific Significance: The gangliosides belong to class of very interesting glycolipids, the structure and distribution of which within the cell is similar to that of the cerebrosides. Analogous to the accumulation of cerebrosides in Gaucher's disease, the gangliosides are known to accumulate in the CNS of children afflicted by Tay-Sach's disease. Study of the anabolic and catabolic pathways affecting ganglioside synthesis and breakdown may increase our understanding of the lipodystrophic diseases.

Proposed Course of Project: The objective will be to establish the composition of the various gangliosides and their constituent building blocks. A study will be made of the distribution of the gangliosides within various species, and it is proposed to investigate their exact localization within the cell by means of immunochemical techniques.

Part B included: No

Serial No. NINDB-NC-18

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Glycolipids as Receptor Sites

Principal Investigator: Eberhard G. Trams

Other Investigators: Robert A. Resnik and Richard L. Irwin

Cooperating Units: Section on Ophthalmology Chemistry, NINDB and Section on Clinical Applied Pharmacology, NINDB

Man Years:
Total: 1.6
Professional: 1.0
Other: 0.6

Project Description:

Project: A study of the possible function of glycolipids as cell receptors.

Objectives: To attempt to identify certain macromolecular glycolipids with cell receptors in brain.

Methods Employed: Glycolipids of the ganglioside class are isolated from various sources and subjected to simultaneous chemical and physical analyses in order to identify shape, size and charge of the molecule. Interaction of these materials with quarternary amines and other CNS-active compounds is studied by bio-assay on isolated organs and in whole animals.

Major Findings: The gangliosides and other similar materials behave in many respects as high-molecular polyanions and have chemical and physical properties which would make them ideal functional membrane constituents. It was found that in small amounts these materials induced repetitive firing in single nerve fibers. It was further observed that these glycolipids seem to be able to sequester a number of quarternary ammonium compounds such as curare, and thus apparently exerted a blocking action on some of these compounds in biological systems. The effect of mucolipids on polyvalent cationic dyes was similarly investigated. The marked metachromasis induced by gangliosides in some of these dyes could be utilized

Part A, continued

for the identification of active groups within the polymer. Some of these results were received at a symposium of the American Academy of Neurology (E. G. Trams; "Galactolipids and Cell Receptors," Miami, April 1960).

Scientific Significance: It would be of paramount importance to chemically identify some of the receptor sites within the CNS and to elucidate their mode of action. Most of the available evidence points to the mucoproteins, mucolipids or closely related materials as cell receptors.

Proposed Course of Project: To investigate the physical-chemical behavior of the polymeric glycolipids and of some derivatives which can be prepared by enzymatic or chemical degradation. It is proposed to study the effect of such fractions in various biological systems such as isolated heart and single nerve fibers.

Part B included: No

Serial No. NINDB-NC-19
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of Hydrocarbons on Enzyme Synthesis

Principal Investigator: Eberhard G. Trams

Other Investigators: Joseph K. Inscoe and Robert A. Resnik

Cooperating Units: Section on Pharmacology, Laboratory of Clinical Science, NIMH and Section on Ophthalmology Chemistry, NINDB

Man Years
Total: 0.4
Professional: 0.3
Other: 0.1

Project Description:

Project: To investigate the effects of carcinogenic hydrocarbons on the synthesis of microsomal enzymes.

Objectives: To ascertain the mode of action of a number of carcinogenic hydrocarbons which have been shown to induce the synthesis of certain microsomal enzyme systems and had an apparent simultaneous inhibitory effect on other enzyme systems.

Methods Employed: Animals received a number of hydrocarbons either in the diet or by intraperitoneal injection. The soluble and particulate fractions from liver homogenates were isolated and assayed for enzymatic activity by standard techniques.

Major Findings: It was observed that an intraperitoneal injection of 3-methyl cholanthrene or benzpyrene markedly stimulated the activity of the glucuronyl transferase and hydroxylases, while a system which can N-dealkylate morphine was inhibited. This effect is apparent within 4-6 hours after administration of the hydrocarbon. In a chronic study, the effect of dietary N-2-fluorenylacetamide and of p-dimethylaminoazobenzene on seven different

Part A, continued

hepatic enzymes was observed. The principal finding was that there was a progressive decline in activity of the N-demethylating system and of the hydroxylating activity of the liver microsomes. The glucuronyl transferase, glutathione reductase and UDPG-dehydrogenase systems appeared chronically stimulated. There were few if any changes in the activity of tryptophan peroxidase and cortisone reductase.

Scientific Significance: These studies were designed to help elucidate the mechanism by which the activity of certain microsomal enzymes were controlled. Although most of the hydrocarbons tested essentially are inducers of neoplastic transformations, it is hoped that this investigation will contribute to the understanding of the so-called "enzyme deletion hypothesis" which might have a general application in the molecular disease concept.

Proposed Course of Project: It is proposed to continue this investigation with the emphasis of possible changes in the lipid membrane of the microsomal particle which might occur during the induction or deletion process.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Conney, A. H., Gillette, James R., Inscoe, Joseph K., Trams, Eberhard G., and Posner, Herbert S. Induced synthesis of liver microsomal enzymes which metabolize foreign compounds, Science, 130: 1478-1479, 1959.

Serial No. NINDB-NC-20
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Carboxylation of Acyl-CoA Derivatives

Principal Investigator: Roscoe O. Brady

Other Investigators: None

Man Years
Total: 1.0
Professional: 0.3
Other: 0.7

Project Description:

Project: Investigation of the enzymatic carboxylation of acetyl-CoA.

Objectives: To learn the mechanism of the biosynthetic pathway for the formation of malonyl coenzyme A and related compounds.

Methods Employed: Radioisotopic techniques in conjunction with standard chromatographic and other analytical techniques are utilized in the identification of the reaction products. Standard procedures are used for the preparation and purification of the enzyme systems involved.

Major Findings: Following the identification of malonyl CoA as a key intermediate in fatty acid synthesis in this laboratory, a program was initiated for the purpose of identifying the exact mechanism by which malonyl CoA was formed. The enzymes isolated from liver and heart which catalyzed the carboxylations in the presence of ATP and bicarbonate were shown to be biotin dependent. Evidence was obtained which indicated that the carboxylation of acetyl CoA to malonyl CoA proceeded possibly through an as yet unidentified intermediate. It was further observed that pyruvate or phosphoenol pyruvate stimulated the carboxylation reaction and that manganese was required. Even with a reasonably pure enzyme preparation, the carboxylation of propionyl CoA proceeded at a much faster rate than the reaction with acetyl CoA even

Part A, continued

though the requirements for the two reactions differed in some respects. Attempts were made to separate the propionyl CoA carboxylase from the acetyl CoA carboxylase.

Scientific Significance: The formation of malonyl CoA from acetyl CoA in a soluble enzyme system is of major importance in the synthesis of long chain fatty acids. An understanding of the reaction mechanism involved will contribute markedly to the understanding of the carboxylation reactions in general.

Proposed Course of Project: It is proposed to purify the enzyme sufficiently in order to elaborate the co-factor requirements and to possibly separate the activity which carboxylates acetyl CoA from the enzyme which carboxylates propionyl CoA. A study will be made of the distribution of the enzymes within the various tissues of the body.

Part B included: No

LABORATORY OF NEUROPHYSIOLOGY

Wade H. Marshall, Chief

Investigators in the Laboratory of Neurophysiology, NIMH-NINDB, are working on a great variety of fundamental problems of the nervous system. Problems range from the initiation of the nerve impulse to social behavior of the South American squirrel monkey (Saimiri sciureus). Material used extends from the "simple" plant cell of Nitella, which reacts with an action potential resembling a nerve cell, to the whole brain of the monkey.

The Section on Special Senses, led by Dr. Ichiji Tasaki, has continued work of fundamental importance on the analysis of the excitable membrane. The squid nerve has been exploited with most ingenious experiments. Tracer work with experiments designed and interpreted on the basis of irreversible thermodynamics was done on both animate and inanimate systems. It was found that the squid nerve membrane was far more permeable to cations than to anions. This fact was related to the fixed negative charge hypothesis originally advanced by Michaelis and Teorell (the latter was a collaborator in this research in 1959). This theory predicates a membrane containing internal negative charges which should tend to attract cations. Following up the consequences of this concept, Dr. Tasaki hypothesized that divalent cations such as Ca^{++} or Mg^{++} should abolish a prolonged action potential (upper stable state of Tasaki) and that the reaction should be endothermic.

In a most effective series of experiments, Dr. Spyropoulos, using the single node preparation of the toad nerve, produced conclusive evidence for such a theory. Delivery of a small amount of Ca^{++} ion to a node in the upper stable state immediately returned it to the lower stable state, i.e. abolished the action potential and returned the membrane to the resting state. Since the displacement of K^{+} ion by Ca^{++} is an endothermic reaction, it was hypothesized that sudden heating of a node would accomplish the same effect. This was found to be true. Conversely, release of Ca^{++} ion from the negative sites in the membrane should be exothermic and a sudden pulse of cooling was found to initiate an action potential in a resting membrane. This is in impressive agreement with the theory, or at the very least, constitutes reasonable grounds for further work along this line of approach. According to Dr. Tasaki's theory the membrane in the resting state is locked in because the divalent Ca^{++} ions successfully compete with K^{+} ions at the negative sites in the membrane.

The movement of Na^+ and K^+ ions which accompanies and follows the action potential has been further studied with tracer technics using the fractionator. The fractionator was first introduced by these investigators in 1959. This important tool has been further developed and now occupies a secure place in the analysis of the nerve impulse. With this device it is possible to get time distributed samples, with a time resolution as short as 25 msec., of tracer bearing wash fluid from a segment of nerve. By prolonging the nerve action potential it is theoretically possible to ask the question: Is there really a time succession of Na^+ flowing in and K^+ flowing out of a nerve during the rising and falling phases of the action potential? Results so far obtained indicate that both K^{42} and Na^{24} flow out of the axon both at beginning and end of the action potential. Further development of the fractionator toward shorter time resolution and greater sensitivity is proceeding. These experiments have been extended to a primitive plant cell (Nitella) which also exhibits an action potential.

These researches have gone far to elucidate general properties of excitable membrane and to permanently change our conceptions of the nerve impulse.

Three visiting scientists, Dr. A. Arvanitaki-Chalazonitis, N. Chalazonitis from France and Dr. T. Oikawa from Japan collaborated in these researches at the Marine Biological Laboratory, Woods Hole, Massachusetts.

The Section on the Spinal Cord, led by Dr. Karl Frank, has continued in important work on fundamental analysis of the nervous system. The investigation of the roles of the principal parts of the neuron, axon and axon hillock, soma and dendrites has been extended. The technic employed (described in last years report NINDB-NP-SC-3) permits activation of a single neuron in a large population. This allows examination of the electric field around the neuron. It was found that the fields for the two main features, the A and B spikes, were not coextensive and further that the B spike was followed by a positive afterpotential. Dr. W. Rall, Office of Mathematical Research, NIAMD, has been a valuable collaborator on this project. His computations of external fields of this type not only are substantial creative contributions, but are also in excellent agreement with the experimental data.

Dr. T. Oikawa, Visiting Scientist, has investigated a large nerve cell in the goldfish. The data strongly suggest that the antidromic impulse does not invade the soma and dendrites. No

evidence was seen of separate A and B spikes common to many other types of cells.

Drs. P.G. Nelson and S.D. Erulkar have been investigating basic mechanisms of hearing in the auditory system at the collicular and geniculate level in the brain. They have observed both inhibitory and excitatory processes in the same cell depending on which ear was stimulated. One of the fascinating problems of hearing is the fact that the human can discriminate time differences of 10 μsec. "between the ears". Studies of this type will eventually explain how times of arrival to the two ears can be analyzed to this level of precision

Drs. Frank and Nelson have undertaken an ambitious but not unrealistic study of the fundamental mechanisms of learning. One of the hypotheses of learning involves the anatomical and functional organization of synaptic connections. In this project methods are employed which permit long time activation of certain pathways to the spinal cord neurons. After periods of time the physiological characteristics of neurons so activated will be compared with control neurons on the opposite side of the spinal cord. The time has come for such developments and Dr. Frank has other projects under consideration in this field of study. It is particularly pertinent that this Section go into this area of research because of the broad and detailed experience acquired in the past decade.

The Section on Membrane Physiology's program is directed into the general area of the ubiquitous excitable membrane and is currently investigating muscle fibers. The surface membrane of both nerve and muscle fibers is electrically excitable, i.e., a wave of self-propagating electrical activity travels along it. Ionic currents carried by sodium and potassium movements are responsible for this activity and the mechanism of electrical excitability has many features common to nerve and muscle. In muscle, however, there is an additional mechanism for the passage of potassium into or out of the cell. This mechanism is not necessary for the production of electrical excitation. An explanation of the additional passage of potassium that evokes the submicroscopic internal tubular structure of muscle is in accord with many of the experimental observations. The tubules could be the pathway for the additional passage for potassium. Such a system could relay the electrical activity from the surface membrane of a muscle fiber to the internal trigger areas for contraction. Some mechanism of this sort is necessary to activate

the internal contractile process with only a short delay after the electrical activity at the surface. The one considered here is admirably suited for this purpose. The work of the Section on Membrane Physiology is to investigate these intriguing fundamental speculations.

The Section on General Neurophysiology has been involved in intracellular analysis of the pyramidal cells of hippocampus, patterning of somatic sensory impulses in cortex and thalamus, work on direct electrical initiation of the cortex and correlation of slow electrical changes and pH of the cerebral cortex.

The hippocampal project has been pursued in a brilliant and energetic way by Drs. Eric Kandel and Alden Spencer for a period of over two years. They have worked out important constants of the pyramidal cell and secured much information on synaptic activation and reaction properties of the pyramidal cell. This work was extended to a study of convulsive seizure patterns. Drs. Kandel and Spencer have received unusual recognition for men of their age. They have been invited to famous laboratories and have also accepted invitations to present their work in a symposium to be published in "Epilepsia", and a presentation to a symposium entitled "Current Problems in Electrobiology" conducted by the American Association for the Advancement of Science and the New York Academy of Sciences.

Dr. Barbara Renkin has proceeded with analysis of sensory patterns, relating cortex and thalamus. This academic year she is a Fellow of the National Science Foundation and is on leave from this laboratory and is working with the distinguished neurophysiologist, Professor Granit at the Karolinska Institute in Stockholm.

Mr. Anthony Bak is developing a brilliantly designed averager for retrieving data from noise. The final model is not yet completed, so final judgment cannot be made at this time. It will be used by Drs. Frank and MacLean and others. Mr. Bak has also given valuable collaboration to several projects in the laboratory Among these is his introduction of the cathodyne, which was crucial in Dr. Spyropoulos' project on the control of nerve impulse in single fibers by chemical and thermal manipulation.

Other work in this section is proceeding on specific relation of pH changes in cortex and spreading cortical depression of Leao. There is also a general project underway on cortical reactions involving analysis of dendritic and soma activity using implanted electrodes.

The Section on Limbic Integration and Behavior, led by Dr. Paul D. MacLean, is very busy with important contributions to the general area of brain and behavior. The squirrel monkey

from South America is the principal experimental animal used by this laboratory. Further developments of technics for employment of this monkey have been made and the stereotaxic brain atlas, (project M-NP-LI-9, 1959) is approaching completion. The work of this section uniquely combines studies of neuroanatomy, neurophysiology and behavior.

Drs. Gergen and MacLean are proceeding with general physiological anatomical analysis of the hippocampus. Under chlorolose anesthesia they have found visually evoked reactions. Search for representation of other exteroceptors in this primitive part of the brain will be made.

The study of convulsive reactions in the hippocampus and effect of these reactions on other parts of the brain and, in turn, on total behavior has been continued. It is known that the hippocampal part of the limbic system is peculiarly sensitive to convulsions and convulsive like reactions. These studies of mechanisms of the total system reactions are of great importance for understanding brain and behavior.

Work on localization of genital function has been continued with the monkey. Dr. MacLean is the first investigator to systematically demonstrate and study the direct physiological and anatomical loci in the brains of animals, electrical and chemical activation of which produced specific sexual responses. This important work is proceeding and an entirely new chapter is being developed in knowledge of brain. As an important example, it has been found that electrical stimulation of the anterior thalamic group produces penile erection. This is specific for the medial part of the medial dorsal nucleus. This nuclear group is directly connected to the phylogenetically new frontal lobes. This constitutes the first proof of a specific anatomical and functional relation of the frontal lobes and a primitive function of the "old" limbic system. This mechanism probably explains some of the behavioral changes observed in patients after frontal lobotomy.

As an important corollary to all work in this section are studies of individual and social behavior of the squirrel monkey.

The senior scientists in the laboratory have received many invitations to present their work at scientific and educational meetings. This shows that the work of the laboratory is respected and is at the very least accepted as well as can be expected of current research.

Serial No. NINDB-NP-SS-5
1. Lab. of Neurophysiology
2. Special Senses Section
3. Marine Biological Lab., Woods Hole, Mass. and Bethesda, Md.
4. Combination of 1a, 3 and 4, 1958.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The mechanism of nerve excitation.

Principal Investigators: I. Tasaki, C. S. Spyropoulos, T. Oikawa, A. Arvanitaki-Chalazonitis and N. Chalazonitis.

Other Investigators: M. E. Ezzy and G. Hayward

Cooperating Units: Dept. of Physiology, Tohoku University Medical School, Sendai, Japan; C.N.R.S., Paris, France.

Man Years (calendar year 1960):
Total: 5
Professional: 3.0
Other: 2.0

Project Description:

Objectives: For a number of years, the primary objective of the Section on Special Senses has been the elucidation of the mechanism of excitation. The process of excitation and its consequence, conduction, constitute the primary physiological event in the nervous system. In addition, it is involved in many other well known biological phenomena such as receptor activity, muscle contraction, cell division and amoeboid movement. Experimental attempts during the last two years at solution of this problem were spurred by collaboration of members of this section with outstanding scientists from other institutions, both in the United States and abroad.

Methods Employed: The method of intracellular multiple wire electrodes, the method intracellular injection and the method of perfusion of the intracellular compartment were employed with the squid giant axon. Standard radioactive tracer techniques and the method of Obrink and Ulfendahl (for

Part A. Proj. Desc. (cont.)

simultaneous measurement of radioactive Na and K) were employed in conjunction with electrophysiological techniques to study tracer movements in the squid giant axon. These same radioactive tracer techniques in conjunction with a cation exchange membrane from Ionics, Inc. were employed to study tracer movements in artificial membranes. A rotating fractionator was employed for measuring tracer movements during the action potential.

Major Findings:

(1) Movements of tracers across membrane in general. The principles governing the movements of tracers across animate and inanimate membranes and their relation, to the movement of the untagged species were worked out on the basis of thermodynamics of irreversible processes. Experimental justification of the proposed principles was obtained by the study of movements of tracers across cation exchange resin membranes. The physical information obtainable by the tracer technique was clarified.

(2) Movement of tracers across the squid axon membrane. The radioactive tracers employed include K^{42}, Na^{24}, Ca^{45}, P^{32}-labelled phosphate, Cl^{36}, S^{35}-labelled sulfate, tritiated water, Cs^{134} and various C^{14}-labelled organic compounds. In the resting state of the squid giant axon, the time constant of loss of intracellular H_2^3O is 0.5 - 1 min. The corresponding figure for Ca^{45}-ion is approximately 20 min. Thiourea and urea (C^{14}-labelled) have the time constant of 30 to 100 min. For all the univalent cations examined (Na^{24}, guanidinium, choline, Cs^{134} and K^{42}), the time constant was between 5 and 15 hr. For anions examined (chloride, sulfate and phosphate) and for large neutral molecules (sucrose and starch), the time constant was larger than 50 hr. In calcium, choline and phosphate, there was chemical binding of the tracer with the axoplasm and probably with the membrane.

The movement of the chemically unbound cationic tracers (Na, K, Cs and guanidinium) is accelerated by repetitive stimulation of the axon. A reduction of the calcium and magnesium concentration or an increase in the potassium concentration in the bathing fluid was found to increase the rate of loss of these unbound cationic tracers. None of the neutral and anionic tracers were found to be affected by

repetitive stimulation or by potassium depolarization. Based on these experimental findings, it was postulated that the resting squid axon membrane has fixed negative charge and that the membrane remains negatively charged during activity.

By the use of a rotating fractionator, it was possible to collect samples of sea water flowing by the squid giant axon at different intervals during the action potential. Preliminary results indicate that both K^{42} and Na^{24} flow out of the axon both at the beginning and at the end of a prolonged action potential. At neither time did there appear to be any profound preference of one ion over the others.

(3) Initiation and abolition of the action potential by chemical and thermal pulses. In the toad single node preparation, treated with Ni^{++}, it was found possible to initiate, in an all-or-none manner, an action potential by application of a small amount of K^{+}-rich fluid. Application of Ca^{++}-rich fluid to the node during the plateau of prolonged action potential was found to abolish the action potential. A pulse of temperature rise applied during the plateau was also found to be capable fo abolishing the action potential. Based on these and other experimental findings, it was concluded that competition between univalent and divalent cations at the negative sites in the membrane plays an essential role in realization of the two stable states of the nerve membrane.

(4) Other related developments.

a) Tracer movement in Nitella. Using the method of rotating fractionator developed for the squid axon, the relationship between the process of action potential production and the membrane permeability was investigated in the Nitella cell. The time course of the loss of intracellular K^{42} during activity was found to be roughly parallel to that of the impedance loss associated with the action potential. The movement of Na^{24} was tested by the same technique without success. It was suggested that the major portion of the intracellular Na^{24} is in a bound form. The influx and efflux of Cl^{36}-ion was carefully investigated in two species of Nitella, N. flexilis and N. megacarpa. It was concluded that this anion is not involved in the primary

step of action potential production.

(b) Physiological interaction between TEA-treated and normal portions of the squid axon membrane. Uniform intracellular injection of TEA (tetraethylammonium chloride) along a long stretch of the axon is known to prolong the action potential from its normal value (approximately 1 msec) up to 100 msec. or more. When a short portion of an axon was treated with a TEA solution, there was only a slight prolongation in the action potential. This was attributed to the effect of an electric current flowing between the normal (untreated) and the TEA-treated portions of the membrane, bringing about premature termination of the action potential. The process of abolition of the action potential was found to propagate along the axon. The mechanism of initiation of repetitive firing of impulses at the boundary between the TEA-treated and normal zone was clarified.

(c) Demonstration of two stable states in Aplysia ganglion cells. Ganglion cells were found whose membrane potential for intrinsic reasons stabilized periodically at the active state for several seconds. The potential was abolished by inhibitory impulses that periodically impinged the cell. The effect of the inhibitory potentials could be simulated by passage of an inward current.

(d) Space clamp of the Nitella internode. A method was developed for insertion of 2 metal wires into the sap of the internode. The action potential was thereby recorded under space clamp conditions. Comparable techniques were employed for insertion of a longitudinal pipette for injection of various chemicals.

(e) Preparation of review entitled "Excitation phenomena and stability of the nerve membrane". During the last year a review was written of our concepts on excitation. This is to be submitted to the Physiological Reviews.

Significance to Neurology Research: This investigation is expected to contribute to the understanding of the normal function of the nervous system.

Proposed Course of Project: It is our opinion that exploitation of the purely electrophysiological approach to

Part A. Proj. Desc. (cont.) Serial No. NINDB-NP-SS-5

the problem of excitation is nearly complete. At this time, we feel that the more promising avenues of research are those utilizing chemical, spectroscopic and mechanical or thermal transducer techniques. To this end, we intend to study, in the next few years, the process of excitation with such tools as interference microscopy, NMR spectroscopy, EPR spectroscopy, ultraviolet and visible light spectroscopy. Furthermore, we plan to make an attempt to measure mechanical and thermal changes during the action potential. Professor Teorell from Sweden has had experience with some of these techniques in the past. We are contemplating inviting him to collaborate with us on this general problem. Three years ago, we collaborated with Dr. Richard Sands at the University of Michigan on EPR spectroscopy. We plan in the next year to resume this study.

Part B included Yes No

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications — Serial No. NINDB-NP-SS-5

Publications other than abstracts from this project:

Tasaki, I. Resting and action potentials of reversed polarity in frog nerve cell. Nature 184: 1574-1575, 1959.

Spyropoulos, C. S. Cytoplasmic pH of nerve fibers. J. Neurochem. 5: 185-194, 1960

Spyropoulos, C. S. and Tasaki, I. Nerve excitation and synaptic transmission. Chapter in: Ann. Rev. Physiol. 22: 407-432, 1960.

Tasaki, I. Nervous activity in abnormal media. Chapter in: Abnormal Nervous Function, Progress in Neurobiology, (Grenell, R.S., ed.), (in press).

Tasaki, I. A thermodynamic treatment of radio-tracer movement across biological membranes. Science (in press).

Tasaki, I., Teorell, T. and Spyropoulos, C. S. Movement of radioactive tracers across squid axon membrane. Am. J. Physiol. (in press).

Spyropoulos, C. S. Initiation and abolition of the electric response of the nerve fiber by thermal and chemical means. Am. J. Physiol. (in press).

Tasaki, I. and Spyropoulos, C. S. Excitation phenomena and stability of the nerve membrane (in preparation).

Honors and Awards relating to this project:

Dr. Tasaki was invited to participate in the Symposium on Abnormal Nervous Function, Boston, Mass., June, 1960.

Dr. Tasaki was invited to participate in the Ciba Foundation Study Group No. 5, "Regulation of the Inorganic Ion Content of Cells", London, England, April, 1960.

Dr. Tasaki was invited to speak at the University of Chicago on "Tracer Movements Across Squid Axon Membrane", January, 1960.

Part B: Honors, Awards and Publications

Serial No. NINDB-NP-SS-5

Honors and Awards relating to this project (cont.):

Dr. Spyropoulos was invited to speak at the Upstate Medical Center of the State University of New York, Syracuse, New York, November 1960, on "The Excitable Membrane".

Serial No. NINDB-NP-SC-3
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. Continuation

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Generation of impulses in nerve cells.

Principal Investigators: K. Frank and P. G. Nelson

Other Investigators: K. Naka (Visiting Fellow) and M. Becker

Cooperating Units: Dr. W. Rall of the Office of Mathematical Research of NIAMD has provided important theoretical assistance.

Man Years:
Total: 3.3
Professional: 2.4
Other: 0.9

Patient Days: None

Project Description:

Objectives: The study of basic physiological mechanisms operating in single nerve cells. Specifically this is a project to determine which parts of the neuron contribute the various components of the recorded potentials, the time sequence of their activation and the quantitative aspects of the changes each part undergoes.

Methods Employed: Previous studies of intracellular potentials, giant extracellular potentials and experiments applying voltage clamp techniques to the motoneuron have yielded ambiguous results with regard to the role played in spike generation of the various cell parts. In an attempt to shed more light on this problem, maps have been made in three dimensions of the external potential fields produced in the spinal cord by activity of a single motoneuron. The axon of such a cell is isolated by a new technique which permits its selective excitation. Extracellular potentials produced by this cell at a distance may be smaller than the random electrical activity of other cells and electrical noise generated by the microelectrode and amplifiers. Techniques augmenting the signal-to-noise

ratio were employed as developed in Project No. NINDB-NP-SC-6, 1959. Analyses have been made of the complex augmented potential transients so recorded.

Major Findings: Potential maps have been prepared for both the compound AB spike and the simpler A spike of the spinal motoneuron. The latencies of the peaks of both A and B components increase with distance from the cell. The apparent conduction velocity of these waves is the same within the limits of precision of the experiment. Fatt had formerly observed this increasing latency for the B spike and had interpreted it as indicating active conduction in the dendrites. However, since the A spike is almost certainly not conducted in the dendrites and has the same apparent conduction velocity, Fatt's conclusion is invalidated. The more probable explanation is that this indicates the conduction velocity of decrementing or simple electrotonic spread over the dendritic tree. Peaks of both A and B spikes are nearly synchronous over a region of about 50-100 microns for the A and 100-200 microns for the B. These regions are roughly centered about the respective maxima of the potentials recorded. However, the two regions of synchrony are not coextensive but are displaced by about 200 microns in the dorsal-ventral direction. The B spike is followed by a positive after potential while the A spike is not.

Dr. W. Rall (Project No. NIAMD-122, 1959) has computed the external fields to be expected in a volume conductor such as the spinal cord from several hypothetical models. The experimental results of this project are in good agreement with Rall's potential fields based on the assumption that antidromic spike activity dies out in the soma or bases of the dendrites. Rall's work indicates that around a structure such as the motoneuron a predominantly negative signal would be recorded from a region much larger than that of the active tissue and that corresponding positive sources would be confined to very small regions about the dendrites. Experimental results of this study verify his prediction; only predominantly negative spikes were recorded. Finally, it has been shown that an after positivity recorded extracellularly does not necessarily indicate that active conduction has proceeded beyond the nearest active membrane--another popular misconception.

Significance to Research: The nature of the activity of the dendrites is an important key to their role in the

Part A. Significance to Research (cont.)Serial No.NINDB-NP-SC-3

integration of excitatory and inhibitory synaptic activity reaching the cell. It is also hoped that these results may elucidate some of the mechanisms of repetitive impulse generation in neurons.

Proposed Course of Project: Last year voltage clamping techniques applied to spinal motoneurons were discontinued pending verification of some of the fundamental assumptions made necessary for their interpretation. It is planned to apply these techniques to the giant nerve cell of the Aplysia during the last half of 1961. This work will be performed at a marine station in Arcachon, France, in collaboration with Dr. Ladislav Tauc, a member of Professor A. Fessard's neurophysiology laboratory in Paris.

Part B included Yes X No ___

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications Serial No.NINDB-NP-SC-3

Publications other than abstracts from this project:

Frank, K. and Fuortes, M. G. F. Accommodation of spinal motoneurones of cats. Arch. ital. Biol., 98: 165-170, 1960.

Frank, K. and Becker, M. C. Microelectrodes for recording and stimulation. In Physical Techniques in Biological Research. (W. L. Nastuk, ed.) New York, Academic Press, Vol. 4 (In press).

Frank, K. and Fuortes, M. G. F. Excitation and conduction. Ann. Rev. Physiol. (In press).

Serial No. NINDB-NP-SC-8
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. Continuation

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Generation of impulses in the Mauthner cell of the goldfish.

Principal Investigator: T. Oikawa (Visiting Scientist)

Cooperating Units: None

Man Years:
Total: 0.5
Professional: 0.5
Other: 0

Patient Days: None

Project Description:

Objectives: To study the electrophysiological mechanisms operating in the large Mauthner cell of the goldfish. While this is a highly specialized nerve cell, it lends itself readily to electrical measurements and may provide a model for other less accessible nerve cells.

Methods Employed: Intra- and extracellular action potentials are recorded during anti- and orthodromic stimulation. Membrane excitability is measured by direct excitation through a glass micropipette. Marking techniques and histological studies indicate the relationships of the extracellular potential fields to the cell structure.

Major Findings: The medulla oblongata of the teleost contains one pair of giant nerve cells, the Mauthner cells, consisting of two long dendrites and an axon passing down the contralateral spinal cord to the tail muscle on the opposite side. Stimulation of this axon near the tail causes antidromic excitation of the opposite Mauthner cell and inhibition, presumably via axon collaterals, of the Mauthner cell on the same side. The multiphasic potential fields produced by the firing of this single cell have been studied and maps made. Reconstructing the activity in the cell from a study of these fields indicates that the large monophasic negative spike is associated with firing in the axon cap; the positive-negative and monophasic positive

Part A. Major Findings (cont.) Serial No. NINDB-NP-SC-8

potential fields are the result of passive depolarization of the cell soma and dendrites respectively. Accordingly, it seems likely that an antidromic impulse does not invade the soma and dendrites.

Evidence was not seen in the extracellular fields of an inflection point in the spike potential, the A-B break seen in so many other nerve cells. Resting and action potentials were determined by intracellular recording from the cell soma to be 70-80 mV and 30-40 mV respectively.

Significance to Research: Results of these studies on a giant nerve cell are being used as a guide for interpretation of results with smaller cells.

Proposed Course of Project: Dr. Oikawa completed his studies in this laboratory in May, 1960, and has returned to Japan where he plans to continue them. Similar studies on a giant nerve cell in the sea slug, Aplysia, will be initiated in this section in June, 1961. See Project No. NINDB-NP-SC-3.

Part B included Yes___ No X

Serial No. NINDB-NP-SC-9
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of chronic changes in single nerve cells associated with learning.

Principal Investigators: K. Frank and P. G. Nelson

Other Investigators: K. Naka (Visiting Fellow) and M. Becker

Cooperating Units: None

Man Years:
Total: 2.2
Professional: 1.3
Other: 0.9

Patient Days: None

Project Description:

Objectives: Basic mechanisms operating in single nerve cells have been studied for many years with microelectrode techniques both in this and other laboratories. The time seems ripe to apply this technique to the elucidation of possible chronic changes in single cells of the central nervous system associated with altered sensory input.

Methods and Proposed Course of Project: While learning is commonly thought to involve primarily changes in groups of nerve cells, such changes necessarily imply that the individual cells have also been modified even if this only involves changes in their connections. Altered patterns of sensory input are causally associated with demonstrable behavioral changes, but which cells are involved has not yet been determined. It is planned therefore to apply long series of stimuli to peripheral nerves on one side only of chronic spinal cats in an attempt to produce a measurable change in the spinal reflexes on the two sides. If behavioral changes can be produced, the preparations will be examined with microelectrodes to determine which cells have been altered and in what way. It is presumed that it may be necessary to use very young cats and techniques are being explored for the use of perinatal animals. Because

Part A. Methods and Proposed Course of Project (cont.) Serial No. NINDB-NP-SC-9

cell changes may involve the activity of the higher central nervous system, it may be necessary to experiment with unoperated animals. With these animals, implanted electrodes on peripheral nerves might produce excessive pain. Therefore it is planned to apply repetitive joint flexion as a means of producing a steady unilateral sensory inflow. Inflow to the spinal cord from the brain may also be altered in an attempt to demonstrate cellular changes in the cord. This can be accomplished either by chronic stimulation or ablation experiments.

Should it prove practical to make such preparations showing chronic changes of a particular group of cells, it is planned to collaborate with members of the Laboratory of Neuroanatomy and possibly others in the exploration of the nature of these changes. Other approaches to the basic objective will be adopted if initial attempts are unsatisfactory.

Major Findings: Preliminary experiments using trains of stimuli through implanted electrodes are in progress but no results can be reported yet.

Part B included Yes X No___

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications Serial No.NINDB-NP-SC-9

Publications other than abstracts from this project:

Guth, L., Soutter, L., Frank, K., Campbell, J. B. and Lloyd, J. B. Diaphragmatic function following anastomosis of recurrent laryngeal and phrenic nerves. Exptl. Neurol., 2: 251-260, 1960.

Serial No. NINDB-NP-SC-10
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Intracellular recordings from inferior colliculus and medial geniculate in response to auditory stimulation.

Principal Investigators: P. G. Nelson and S. D. Erulkar

Other Investigators: M. Becker

Cooperating Units: Dr. Erulkar is from the Department of Pharmacology of the University of Pennsylvania. He spends every other weekend in this laboratory.

Man Years:
Total: 1.0
Professional: 0.6
Other: 0.4

Patient Days: None

Project Description:

Objectives: To study basic mechanisms operating in single cells at the collicular and geniculate level of the auditory pathway.

Methods Employed: Clicks and tone envelopes are delivered to the cats ears. Hyperfine glass micropipette electrodes are inserted into inferior colliculus or medial geniculate cells and transmembrane synaptic and spike potentials recorded. Responses to various patterns of auditory stimuli are recorded and currents delivered through the micropipette are also used to analyze the nature of the synaptic potentials.

Major Findings:

1. In both colliculus and geniculate both excitatory and inhibitory post synaptic potentials have been seen. Even when the usual burst type responses occur, an inhibitory component to the synaptic activation is usually seen. Polarization of the cell membrane has revealed a direct inhibitory process in both collicular and geniculate cells.

Part A. Major Findings (cont.) Serial No. NINDB-NP-SC-10

2. Some units in the colliculus have been seen to respond to a click in one ear with an EPSP and to a click in the other ear with an IPSP.

3. The interaction of the synaptic potentials from the two ears may provide a precise mechanism for comparing time of arrival of the click at the two ears.

4. AB spikes like those of motor horn cells have been clearly observed in some of the cells penetrated.

5. Potentials similar to those associated with Renshaw firing in the spinal cord have been seen occasionally.

Significance to Research: The intracellular technique has many limitations due to the undoubtedly severe injury that impalement inflicts on a cell. Nonetheless, direct demonstration of the nature of synaptic input to different structures along the auditory pathway may help to understand auditory integrative mechanisms and where they occur.

Proposed Course of Project: It is proposed to extend the analysis of responses to tonal stimuli. Histological control of the penetrations is being carried out at the University of Pennsylvania. It may be possible to demonstrate intranuclear integrative mechanisms such as a feedback system analogous to the Renshaw loop in the spinal cord.

If this work is not completed by the end of 1960, it is planned to continue it on a part time basis.

Part B included Yes___ No_X_

PUBLICATIONS, 1960
INTRAMURAL RESEARCH, NINDB

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Ajmone Marsan, C.: Electrographic aspects of "epileptic neuronal aggregates". Epilepsia, 1961, in press.

Ajmone Marsan, C. and Lewis, W. R : Pathologic findings in patients with "centrencephalic" electroencephalographic patterns. Neurology 10: 922-930, 1960.

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Strang, R. and Ajmone Marsan, C.: Pathological and electroencephalographic analysis of metastatic tumors of the brain. AMA Arch. Neurol., in press.

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Medical Neurology Branch:

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Ophthalmology Branch:

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AWARDS, RECOGNITIONS, AND ACTIVITIES

Staff Appointments to Universities:

Dr. Cosimo Ajmone Marsan, Instructor in Clinical Neurology, George Washington University

Dr. Maitland Baldwin, Associate Clinical Professor of Neurosurgery, Georgetown University

Dr. Anatole S. Dekaban, Assistant Professor of Neurology, George Washington University

Dr. G. Milton Shy, Associate Clinical Professor of Neurology, Georgetown University

Dr. Donald B. Tower, Associate Clinical Professor of Neurology; Associate Professor of Biochemistry; and Research Consultant, Georgetown University

Hospital Consultants:

Dr. Maitland Baldwin, U. S. Naval Medical Center, Bethesda, Maryland

Dr. Anatole S. Dekaban, Children's Hospital, Washington, D. C.

Dr. G. Milton Shy, U. S. Naval Medical Center, Bethesda, Maryland and D. C. General Hospital, Washington, D. C.

Awards and Honors:

Dr. Cosimo Ajmone Marsan, Editorial Board, EEG Journal; Examiner, Board of Electroencephalography

Dr. Milton W. Brightman, President, Washington Society for Electron Microscopy, 1960-61

Dr. Kenneth S. Cole, Editorial Board, Biophysical Journal

Dr. Karl Frank, Associate Editor, Experimental Neurology

Mr. William B. Matthews, Jr., Superior Performance Award, NIH, 1960

Dr. John W. Moore, Editorial Board, Review of Scientific Instruments

Dr. Sanford L. Palay, Editorial Review Board, Journal of Biophysical and Biochemical Cytology; Editorial Board, Experimental Neurology

Awards and Honors (Cont'd):

Dr. Grant L. Rasmussen, NIH Representative, Committee of Hearing and Bio-acoustics, Armed Forces Research Council

Dr. G. Milton Shy, Examiner, American Board of Psychiatry and Neurology

Dr. Donald B. Tower, Chairman, Section on Neurochemistry, American Academy of Neurology; Editorial Board, Neurology, and Biochemical Pharmacology; Member, Neurochemistry Panel of CIOMS (UNESCO-WHO)

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